BRITISH JOURNAL OF CANCER

VOL. IV SEPTEMBER, 1950 NO. 3

PROGNOSIS IN CARCINOMA OF THE BREAST.

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Received for publication May 18, 1950.

In recent years there has been much controversy concerning the treatment of mammary carcinoma. Not only has the type of therapy most likely to achieve success been the subject of dispute, but also the same measures in the hands of well-known authorities have produced a wide variation in results. It seems likely that the difficulties arise from comparing the results obtained in groups of cases which are not strictly comparable. Before the different methods can be assessed adequately we must have a reliable system for classifying patients with this disease. The importance of this is two-fold. Firstly, it provides a more accurate guide to prognosis, and secondly, it ensures the proper grouping of similar cases for comparison. Have we a classification which accomplishes this ?

The outlook for a patient with cancer of the breast depends first and foremost upon the degree of malignancy of the tumour and upon its extent. It is a wide belief that the prognosis is also influenced by certain other features, but the value of these is open to question and will form the subject of a separate inquiry. Suffice here to state that such factors as age of patient, site of tumour in the breast, size of primary growth and duration of symptoms were not found, *per se*, to influence the outlook materially.

How can we determine the degree of malignancy of mammary cancer and also its extent ? The former problem will be discussed presently. The latter is assessed by the results of a clinical examination, and forms the basis of classifications of this disease now in general use. These are undoubtedly of value, but they fail to account for at least a 25 or 30 per cent five-year mortality for growths which are apparently confined to the breast (i.e. Stage 1). In addition, there are also surprising successes achieved for the more advanced cases.

Attempts have been made in the past to group patients with breast cancer according to histological criteria which, it has been claimed, give some indication as to the degree of malignancy of the tumour. Two methods are available. One depends upon the relative amounts of epithelial and connective tissue elements, and gives such types as encephaloid, scirrhus and atrophic scirrhus. The other is based upon a system of grading, Whilst the results of the former have largely proved disappointing, conflicting views exist as to the value of the latter. The problem of grading has been investigated chiefly in the United States.

It has received but little attention in this country, though Patey and Scarff (1928) and later Scarff and Handley (1938) pointed out its importance in carcinoma of the breast.

It is the prime object of this paper to investigate the significance of the grade of malignancy in mammary cancer in an attempt to establish a more accurate system of classifying patients with this disease. It is thus hoped to contribute information which will not only assist in formulating prognosis, but also aid in the search for the best form of treatment.

There is great difficulty at the present time in deciding on the line of action to adopt in view of the widely different procedures advocated by different authorities. The magnitude of the problem facing surgeons and radiotherapists can perhaps be appreciated by considering the divergence of opinion expressed during the course of a single year regarding the management of early cases (i.e. Stage 1). Whilst Gordon-Taylor (1948) and Cade (1948) believe radical mastectomy alone to be the most suitable treatment for these patients, Riddell (1948) and also Richards (1948) advocate ancillary radiotherapy. On the other hand, even the value of the radical operation has been challenged. Keynes (1937) was the pioneer in this country of more conservative measures for breast cancer, and now McWhirter (1948*a*, 1948*b*) has complicated the issue further by making a case for the simple operation with deep X-rays to the undissected axilla.

Breast cancer, by virtue of its acccessibility, would appear to be an example of malignant disease ideally suited for surgical treatment. It is now over fifty years since Moore of London conceived and introduced the radical mastectomy (Rodman, 1904), and still there is controversy in some quarters as to its value. If we cannot solve the problems of mammary carcinoma, what hope is there for the surgery of malignant disease in less accessible sites?

Previous Attempts at Grading.

Von Hansemann in 1893 was the first to point to the relationship between the histological picture and the behaviour of tumours. He introduced the term "anaplasia," which implied a loss of differentiation and the increase of reproductive power of a growth. He concluded in 1902 that, with rare exceptions, the greater the anaplasia, the greater the tendency to form metastases (Plaut, 1927).

Little attention was directed to the grading of tumours until Broders (1920) applied von Hansemann's theory to squamous cell carcinoma of the lip, and a year later to similar tumours of the skin (Broders, 1921). He revealed a close relationship between the degree of anaplasia and the length of survival.

Greenough (1925) was the first to grade cancer of the breast according to the principle of anaplasia. The prognostic value of a number of epithelial features were studied and three grades of malignancy were recognized. A parallel was found between the histological picture and the progress of the patient.

MacCarty and Sistrunk (1922) and MacCarty (1922, 1924) also studied the histology of breast tumours, and found the most important prognostic characteristics to be the degree of cellular differentiation and the presence or absence of certain stromal features.

White (1927) was unable to agree with the views of MacCarty, but in a series of 100 cases confirmed the results of Greenough (1925).

Patey and Scarff (1928), in a small preliminary group of cases, largely followed Greenough's method of classification, and showed a definite correlation between the histology and clinical course in breast cancer. This work was supported by the results of a larger investigation carried out by Scarff and Handley (1938).

Smith and Bartlett (1929), and later Simmons, Wright, Hartwell and Greenough (1933), also confirmed the value of the system introduced by Greenough (1925). The five-year survival rates for the three grades of malignancy obtained by these authors are comparable, and agree in the main with those of Patey and Scarff (1928).

Lee and Stübenbord (1928) considered that too much emphasis had been placed on histological grading. They therefore elaborated a " clinical index of malignancy " based upon age, presence of lactation, rate of growth of tumour, and extent (stage) of the disease. Three groups were established and 100 cases studied. To compare clinical with histological grading Ewing classified the same cases into three grades ; details of his method were not given. The authors concluded that histological grading is less effective in furnishing a prognosis than their clinical plan. Recently Richards (1948) has modified this system and included a factor for site of growth. He claims the method to be the most reliable guide to prognosis available at present. On the other hand, it has not been generally accepted that age and site of tumour are of prognostic significance. Perry (1925), Lane-Claypon (1928), Truscott (1947) and Harnett (1948) were not able to prove that the position of the growth had any bearing on outcome in breast cancer. Similarly, age was not found of value by Haagensen and Stout (1943), Truscott (1947), Harnett (1948) and others. These points, however, will be discussed more fully in a subsequent investigation.

Flothow (1928), in a little over 200 cases of carcinoma of the breast, studied the factors stated by MacCarty (1922) to represent a defensive mechanism by the body to a malignant tumour. His results support the views of MacCarty, and the presence or absence of these factors were considered to be of value in prognosis.

Hueper has gone so far as to devise a " histological malignogram " based upon no less than twenty different features. It was shown that the malignancy increased with a rise in the " malignancy index." For comparison a clinical classification was also elaborated by Schmitz, but no attempt was made to study the effect of correlating these histological and clinical systems (Hueper and Schmitz, 1929).

Opinions regarding the accuracy of histological grading have varied from Hueper, who finds as many as twenty different features of prognostic value (Hueper and Schmitz, 1929), to Reimann (1929), who is unable to find one. The latter, employing a number of histological criteria, tried unsuccessfully to grade 100 cases of breast cancer. He concluded that it is futile to decide from an examination of a section of the tumour what will happen to the patient.

Plaut (1927) reviewed the literature on the grading of cancer, and does not advocate the method of histological prognosis for practical purposes.

More recently Willis (1948) states that attempts at precise numerical histological grading of tumours as an index of their malignancy are very arbitrary, unscientific and wasted effort. He considers, however, the broad grouping methods based on general histological stucture—presumably such as that of Greenough (1925)—to be of value.

Criteria Employed in Histological Grading.

The histological features which have been chosen as the basis for grading breast cancer have varied with different authors. While some have attached chief importance to parenchymal features, others have directed their attention to the stroma; many have combined both elements. MacCarty (1922) found cellular differentiation, hyalinization, fibrosis and lymphocytic infiltration to be of prognostic value. His criteria were employed by Flothow (1928), who reached a similar conclusion. Greenough (1925), on the other hand, applying the principal of anaplasia, concentrated on tubule formation, variation in size of cells and nuclei, secretory activity and mitotic and hyperchromatic figures. He found round cell infiltration and hyalinization of little value in estimating prognosis. White (1927) investigated the factors employed by MacCarty (1922) and by Greenough (1925). His results support the views of only the latter.

The principles laid down by Greenough (1925) were closely followed by others, such as Smith and Bartlett (1929), and Simmons, Wright, Hartwell and Greenough (1933). Patey and Scarff (1928) and Scarff and Handley (1938), however, attached chief importance to tubule formation, variation in size of nuclei, and mitotic and hyperchromatic figures.

Hueper (Hueper and Schmitz, 1929) employed many features in both the parenchymal and stromal elements in his complex grading plan.

Delbet and Mendaro (1927) in France, on the other hand, considered the presence or absence of mucous secretion to be the most important factor in ascertaining the outcome of patients with mammary carcinoma. They used the mucicarmine stain to study this feature. Leroux and Perrot (1928) and Moureau and Lambert (1932) support the views of Delbet and Mendaro (1927), but Betrand and de Nagey (1931) consider muco-secretion to be of no value in determining clinical progress.

Because of the conflicting views regarding the prognostic significance of various histological features in cancer of the breast, Haagensen (1933) investigated 15 different factors separately. They fall into three main groups—manner of growth, cell morphology and reaction of stroma. The author based a grading system on those elements he found to be of prognostic value. These were papillary arrangement, comedo arrangement, adenoid arrangement, variation in size and shape of nuclei, number of mitoses and gelatinous degeneration. Three grades of malignancy were recognized, and were found to bear a close relationship to the period of survival. Haagensen (1933) concluded that histological grading is simply an additional prognostic guide, and should be subordinate to the clinical data.

Evans (1933) studied a number of parenchymal and stromal features, and concluded that, although anaplasia indicates a poor prognosis, it is of no practical value in assessing the outcome for the individual case. His results largely agree with those of Greenough (1925), but number of mitoses was found to be of no significance.

Sophian (1935) also investigated both parenchymal and stromal factors in breast carcinoma. The most important criteria appeared to be adenoid arrangement, constancy of nuclear size, actual nuclear size, mitoses and secretion. Three grades of malignancy were established, and a definite correlation was found between histology and prognosis.

Harrington (1937), in a clinical and pathological study, graded a large series

of breast cancers according to the method of Broders (1920). He concluded that the grade of malignancy is the most important indication of survival period, and this is more significant when considered together with the state of the axillary glands.

From what has been said it is clear that opinions vary regarding the prognostic value of histological grading. Even when a close parallel was demonstrated between microscopic appearance and clinical progress, some workers have hesitated to advocate the method for practical purposes.

Complicated, exact numerical systems, such as the "histological malignogram" introduced by Hueper (Hueper and Schmitz, 1929), are time-consuming and of doubtful value.

Most success in the field of histological grading for breast cancer appears to be with the method of Greenough (1925). This has been employed by several authors, and found to be of definite value in indicating the likely outcome of patients with this disease.

PRESENT INVESTIGATION.

Material.

The series taken for the present investigation consists of 565 cases of carcinoma of the breast treated at the Middlesex Hospital between the years 1936 and 1942 inclusive, and at the war sector units associated with it during the latter half of this period. In spite of the movement of population produced by the recent conflict, all but 11 of the cases were traced by the follow-up department of the hospital. Success, however, was not so great in collecting the histological slides, and in 36 cases they could not be obtained. There were 12 post-operative deaths and 4 air-raid casualties. These 63 cases were excluded, as were a further 32 for various reasons tabulated below. With these deductions 470 patients were left for study.

	Number of cases.
Total	565
To be excluded—	
Untraced	11
Post-operative deaths	12
Air-raid casualties	4
No histological sections available	36
No carcinoma or query carcinoma in sections available	23
Impossible to grade	7
Sarcoma	2
	95
Remaining for consideration	470

It is to be noted that in seven instances grading was not carried out. In these cases the only sections of tissue available were either too small or else prepared by the frozen technique. Grading in such circumstances, it will be pointed out later, is apt to be inaccurate.

Of the total cases, 209 were treated by surgery alone, and 261 by surgery with ancillary radiotherapy. Further details of treatment will be considered

later. It must be emphasized that all patients in this investigation were subjected to some form of surgical procedure. Pure radiotherapy cases could not be considered owing to the absence of biopsy or surgical specimens for grading purposes. It is therefore obvious that the most advanced and hopeless cases have been excluded. Hence, our results are likely to be superior to those of previous reports from this hospital in which these cases were taken into account.

For the present study the five-year survival rate will be the chief prognostic yard-stick, though some attention will also be given to ten-year results. "Survival rate," however, does not necessarily imply freedom from cancer; it is merely an indication of the number of patients actually alive. In point of fact, many of them have obvious, distant secondary deposits.

Method of grading.

The method of grading used by Patey and Scarff (1928) was employed. This was based on the principles laid down by Greenough (1925), but chief importance was attached to tubule formation, regularity in size, shape and staining of nuclei and hyperchromatism and mitoses.

Tubule formation.—Well-marked tubule formation was considered of favourable prognosis. This view is supported by the work of Greenough (1925), White (1927), Patey and Scarff (1928), Smith and Bartlett (1929), Sophian (1935), and Scarff and Handley (1938). In fact, this is the only histological feature on which practically all workers in this field have agreed is of prognostic value.

Regularity in size, shape and staining of nuclei.—The greater the irregularity the worse the prognosis. This was found to be the case by the same group of workers quoted in the previous paragraph. Certain authors, such as Evans (1933), Greenough (1925) and Haagensen (1933), paid attention to variation in size of the cells, but the outlines of these are usually indistinct; it is more reliable to consider the nuclei. This feature is considered one of the best indications of the degree of anaplasia, and therefore of the malignancy of the growth.

Hyperchromatic nuclei and mitoses.—The greater the number present the more gloomy the prognosis. Evans (1933) was unable to support this view, and Plaut (1927) considers the feature unreliable on the grounds that many cancer cells divide by amitosis. Nevertheless, as Haagensen (1933) points out, a considerable proportion of the cells in cancer of the breast divide by mitosis, and he found a close relationship between the number present and the outcome. This is also supported by the work of Greenough (1925), White (1927), Patey and Scarff (1928), Smith and Bartlett (1929), Sophian (1935), and Simmons, Wright, Hartwell and Greenough (1933).

It was noted whether each of the above three factors was absent or present in slight, moderate or marked degree. The tumour was then placed in one of the following grades:

Grade I. Carcinoma of low malignancy.
Grade II. Carcinoma of moderate malignancy.
Grade III. Carcinoma of high malignancy.

Microphotographs illustrating examples of these grades are given in Fig. 1 to 12.

The cases were fairly evenly distributed, 30 per cent of the total being placed in Grade I, 41 per cent in Grade II, and 29 per cent in Grade III. Similar results

were obtained by Greenough (1925), Haagensen (1933), and Scarff and Handley (1938). White (1927), however, placed most of his cases in the intermediate grade. Harrington (1937), on the other hand, using Broder's (1920) method, found the majority of his material falling into the highest grade of malignancy, 51 per cent in Grade IV compared with only 4 per cent in Grade I.

Grading in the Present Series.

Grade and prognosis.

When grading of the cases in the present investigation had been completed the clinical details were obtained from the follow-up department of the hospital. The five-year survival rate for all treatments was determined for each grade of tumour; these are shown in Table I.

TABLE I.—*Grade and Prognosis. 5-Year Survivals (All Treatments).*

Grade.	Cases.	Alive at 5 years.	
		No.	Per cent.
I	141	111	79
II	191	81	42
III	138	35	25
Total	470	227	48

It is evident that a parallel exists between grading and prognosis, there being more than three times the number of patients alive with Grade I than with Grade III tumours.

The present results may be compared with those of other authors who have employed Greenough's (1925) principles in grading (Table II). It will be noted that they agree especially with the findings of Patey and Scarff (1928), and

TABLE II.—*Grade and Prognosis by Various Authors.*

Grade.	5-year survivals according to author.					
	Greenough, 1925. Per cent.	Patey and Scarff, 1928. Per cent.	White, 1927. Per cent.	Smith and Bartlett, 1929. Per cent.	Simmons *et al.*, 1933. Per cent.	Scarff and Handley, 1938. Per cent.
I	68	69	66	87	83	45
II	33	42	47	42	45	29
III	*Nil*	23	*Nil*	14	25	23

Simmons, Wright, Hartwell and Greenough (1933). No Grade III cases remained alive in the series of Greenough (1925), and also of White (1927), but this does not appear to be the general experience.

The total survival rate of 48 per cent obtained here agrees very closely with the results of certain other writers in much larger investigations. Thus, 48 per cent was also given by Harrington (1946) among some 5700 patients at the Mayo Clinic. Richards (1948) quotes 43 per cent for a little over a thousand cases, and Adair (1943), 51 per cent for a similar number.

The mean duration of life in months for cases dying within ten years of operation is shown in Table III. Once again a parallel is demonstrated between grade of malignancy and duration of life, Grade I patients living twice as long as those belonging to Grade III.

TABLE III.—*Mean Duration of Life for Cases Dying within Ten years of Operation.*

Grade.	Cases dead.	Survival in months.
I	41	59·6
II	103	40·3
III	74	30·1
Total	218	43·3

The five-year survival period is a convenient yard-stick with which to gauge prognosis in breast cancer, but as Truscott (1947) and others have pointed out, a large number of fatalities from this disease occur from five to ten years after operation. Hence, surgeons are now becoming more and more interested in longer follow-up periods. For this reason the ten-year results will also be given here. Three hundred and ten cases, treated between the years 1936 and 1939 inclusive, were available for this study. The survival rates for the three grades of malignancy are displayed in Table IV. As with the shorter follow-up a parallel

TABLE IV.—*Grade and Prognosis. 10-Year Survivals (All Treatments)*

Grade.	Total cases.	Alive at 10 years.	
		No.	Per cent.
I	82	41	45
II	144	41	28
III	84	11	13
Total	310	93	30

EXPLANATION OF PLATES.

FIG. 1.—Low malignancy (Grade I). Well-marked tubule formation; nuclei regular in size, outline and staining; absence of hyperchromatic nuclei and mitotic figures. × 45.

FIG. 2.—As for Fig. 1. × 350.

FIG. 3.—Low malignancy (Grade I). Well-marked tubule formation; nuclei regular in size, outline and staining; occasional mitotic figures. × 45.

FIG. 4.—As for Fig. 3. × 350.

FIG. 5.—Moderate malignancy (Grade II). Moderate tubule formation; moderate irregularity in size, outline and staining of nuclei; moderate number of hyperchromatic nuclei and mitotic figures. × 45.

FIG. 6.—As for Fig. 5. × 350.

FIG. 7.—Moderate malignancy (Grade II). Slight attempt at tubule formation; moderate irregularity in size, outline and staining of nuclei; few mitotic figures. × 45.

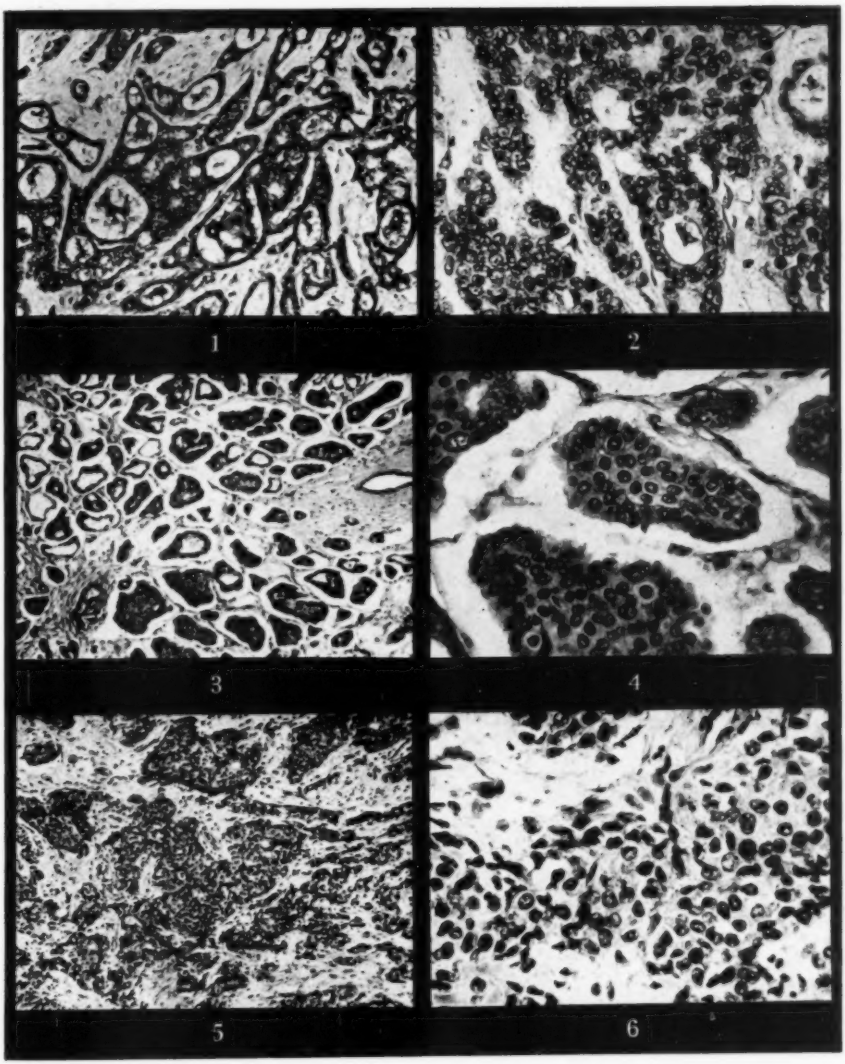
FIG. 8.—As for Fig. 7. × 350.

FIG. 9.—High malignancy (Grade III). No tubule formation; moderate irregularity in size, outline and staining of nuclei; numerous hyperchromatic nuclei and mitotic figures. × 45.

FIG. 10.—As for Fig. 9. × 350.

FIG. 11. High malignancy (Grade III). No tubule formation; marked irregularity in size, outline and staining of nuclei; numerous hyperchromatic nuclei and mitotic figures. × 45.

FIG. 12.—As for Fig. 11. × 350.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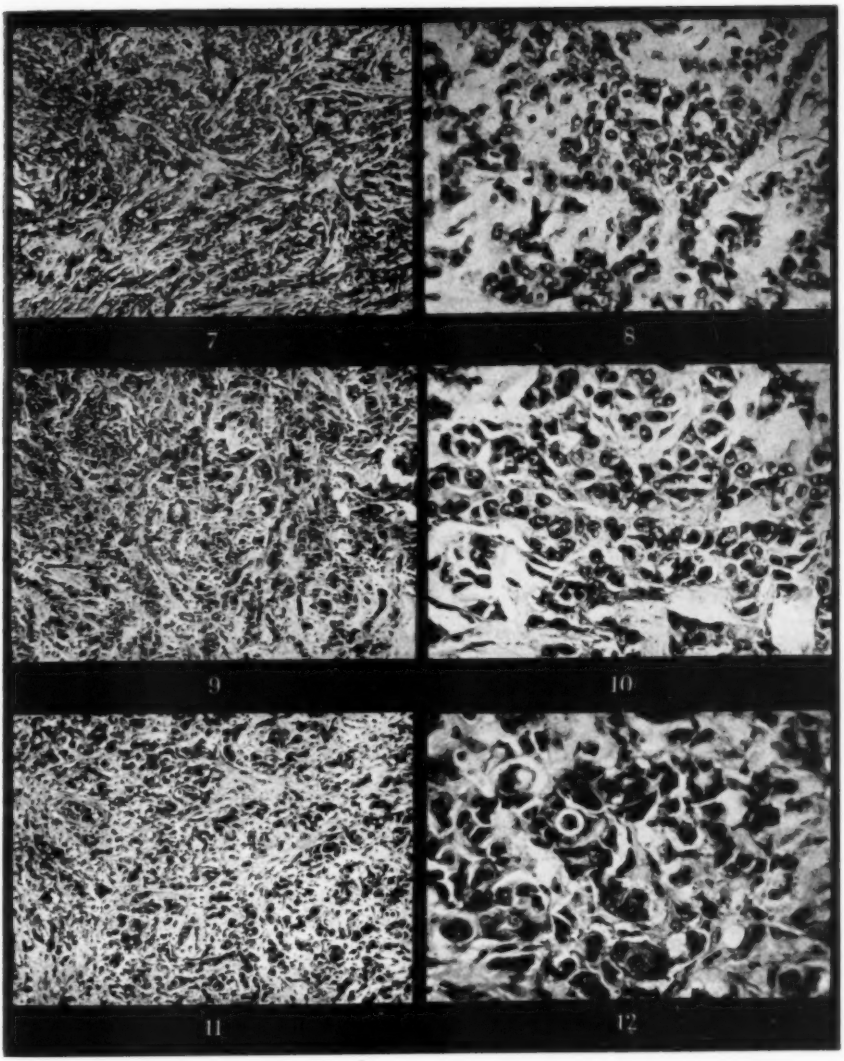
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is again revealed between grade and prognosis, there being between three and four times the number of cases alive with Grade I than with Grade III tumours.

Comparison of the five- and ten-year results emphasizes the importance of the latter as a more accurate indicator of the control of mammary carcinoma, there being a striking fall in the numbers of survivals for the corresponding grades of tumour, even with allowance for expected deaths from other causes.

The ten-year survival rate for all patients was 30 per cent. This is in keeping with the 26 per cent obtained by Richards (1948), and the 32 per cent by Harrington (1946).

Prognostic value of the individual factors employed in grading.

The prognostic importance of each of the three factors employed in grading was investigated separately. The results are set out in Table V. It is seen that well-marked tubule formation is of value in indicating a relatively good prognosis. There appears to be little difference in outcome among the cases with moderate and those with slight or no tubule formation. Variation in size of nuclei and number of hyperchromatic and mitotic figures appear to be of greater value in grading. These results agree on the whole with those of Greenough (1925) and Haagensen (1933), who studied their grading factors separately.

TABLE V.—*Individual Grading Factors and Prognosis.*

Factor.	Degree.	Cases.	5-year survivals. Per cent.
Tubule formation	(i) Well-marked	105	73
	(ii) Moderate	256	42
	(iii) Slight or *nil*	109	39
	Total	470	48
Variation in nuclei	(i) Slight	87	72
	(ii) Moderate	225	53
	(iii) Marked	158	29
	Total	470	48
Hyperchromatic and mitotic figures	(i) Very few or *nil*	73	86
	(ii) Moderate	231	52
	(iii) Marked	166	27
	Total	470	48

Grade, glandular involvement and prognosis.

As various authors have pointed out, it is unwise to rely on grading alone for the assessment of prognosis. The extent of the growth should also be taken into account, and the most important single factor indicating this is whether the axillary lymph nodes are histologically involved or not. In the present material these structures were available for examination in 382 instances. The five-year

survivals for cases with and without this complication are seen in Table VI. It is evident that more than twice the number of patients without glandular spread are alive compared with those whose glands are invaded. This proportion agrees very closely with the one given by Harrington (1937) in over 4000 cases at the Mayo Clinic.

TABLE VI.—*Glandular Involvement (Histological) and Prognosis.*

Axillary glands.	Cases.	5-year survivals.	
		No.	Per cent.
Not invaded	170	121	71
Invaded	212	68	32
Total	382	189	52

The results of combining histological grading with the state of the axillary nodes are shown in Table VII. This procedure clearly enhances the value of grading. The outlook for patients with Grade I tumours without axillary metastases is very different than for those with Grade III growths and this complication (94 per cent. alive at five years in the former group compared with 16 per cent in the latter). It is particularly interesting to note that the prognosis in the lower grade of malignancy with glandular spread is better than for either the higher or intermediate grades without such metastases (65 per cent alive at five years compared with 53 per cent and 61 per cent respectively).

TABLE VII.—*Glandular Involvement, Grade and Prognosis. (All Treatments).*

Axillary glands.	Grade.	Cases.	5-year survivals.	
			No.	Per cent.
Not invaded	I	62	58	94
	II	74	45	61
	III	34	18	53
Total		170	121	71
Invaded	I	43	28	65
	II	92	28	30
	III	77	12	16
Total		212	68	32

These results agree in principle with those of most workers who have employed the system introduced by Greenough (1925), and are almost identical with the findings of Simmons, Wright, Hartwell and Greenough (1933) (Table VIII). Patey and Scarff (1928) and Scarff and Handley (1938), on the other hand, found a uniformly good result in all three grades when the glands were not involved, and so concluded that grading is only of prognostic value in those cases with axillary metastases. The balance of evidence, however, does not appear to support this view.

TABLE VIII.—*Glandular Involvement, Grade and Prognosis (Simmons, Wright, Hartwell and Greenough, 1933).*

Axillary glands.	Grade.	5-year survivals. Per cent.
Not invaded	I	92
	II	62
	III	50
Invaded	I	65
	II	32
	III	20

Relation between histology and glandular metastases.

A parallel has been demonstrated between the histological appearance of breast cancer and prognosis. Can the histology be correlated more directly with the tendency of the growth to metastasize? With reference to this question Table IX has been constructed. It is evident from a study of this table that the proportion of cases with involvement of the axillary lymph nodes increases with the rise in grade of malignancy. The significance of this, however, cannot be accurately assessed without considering the time factor. Thus any type of breast cancer will give rise to axillary spread, provided that it is present in the patient for sufficient length of time. The figures in Table IX refer to the state of the cases at the time of operation, and the time factor involved here is the delay in seeking treatment. This delay was assumed originally to be fairly comparable for each group of patients. On the other hand, if for any reason the Grade III cases tend to present for treatment after a longer interval of time than those belonging to Grade I, then this may account for the greater incidence of axillary involvement in the former. Investigations, however, revealed that this was not the case. On the contrary, the patients with tumours of higher grade malignancy appear to have symptoms of briefer duration as revealed in Tables X, XI.

TABLE IX.—*Cases with Axillary Involvement According to Grade of Malignancy*

Grade.	Cases.	With axillary involvement.	
		No.	Per cent.
I	105	43	41
II	166	92	55
III	111	77	69
Total	382	212	55

TABLE X.—*Mean Duration of Symptoms According to Grade.*

Grade.	Cases.	Mean duration of symptoms. Months.
I	124	10·1
II	174	7·6
III	125	7·1
Total	423	8·3

TABLE XI.—*Distribution of Cases According to Grade and Duration of Symptoms.*

Grade.	Duration of symptoms in months. 6 or less. Cases per cent.	7–12. Cases per cent.	More than 12. Cases per cent.	Total. Per cent.
I	56	21	23	100
II	70	18	12	100
III	69	21	10	100

It is evident that the mean duration of symptoms is shorter for women with Grade III than with Grade I growths. In addition a higher percentage of the former present within six months of the onset of the disease and fewer after twelve months as compared with the Grade I cases. Further attention to this feature will be given in a subsequent paper. Suffice at present to employ the information solely for the purpose of lending weight to the conclusions implied in Table IX. Thus, in spite of the shorter delay in seeking treatment, there is a greater incidence of axillary involvement among the high and intermediate grades of malignancy, consequently emphasizing the greater metastasizing power of these types of growth.

The evidence presented here points to the fact that the degree of malignancy of breast cancer is reflected in its histology. Ewing (1940), however, prefers to employ the term "potential malignancy" for a neoplasm as determined by microscopic examination, because the clinical course may be modified by such features as site of tumour, surgical trauma and perhaps changes in rate of growth. He concludes that with these limitations a close parallel exists between histological structure and malignancy.

Clinical Staging for Prognosis.

Several authors, such as Hueper and Schmitz (1929), and also Lee and Stübenbord (1928), have compared the results of grading and staging in breast cancer. Opinions have varied as to which procedure offers the most reliable guide to prognosis. Let us, therefore, examine the effects of staging in the present series and compare the results with those of the histological classification.

Staging.

Records for assessing the stage which the growth had reached were available in 466 instances. The Manchester Plan was employed, but glandular involvement was determined, where possible, by microscopic examination. The reason for this will be pointed out later.

Clinical staging system (Manchester).

Stage 1.—The growth is confined to the breast. Involvement of the skin directly over and in continuity with the tumour does not affect staging provided that the area involved is small in relation to the size of the breast.

Stage 2.—As for Stage 1, but there are palpable mobile lymph nodes in the axilla.

Stage 3.—The growth is extending beyond the corpus mammae, as shown by—

(*a*) The skin is invaded or fixed over an area large in relation to the size of the breast.

(*b*) The tumour is fixed to underlying muscle. Axillary glands may or may not be palpable, but if glands are present they must be mobile.

Stage 4.—The growth has spread beyond the breast area, as shown by—

(*a*) Fixation of axillary glands indicating extension outside the capsule.

(*b*) The tumour is completely fixed to the chest wall.

(*c*) Secondary deposits in the supra-clavicular lymph glands.

(*d*) Secondary deposits in the skin wide of the tumour.

(*e*) Secondary deposits in the opposite breast.

(*f*) Distant metastases, e.g. bone, liver, brain, lung, etc.

The five-year survivals according to stage are shown in Table XII. For comparison the ten-year results are also given (Table XIII). As previously, when the cases were classified according to grade (Tables I, IV), a large number of deaths are seen to take place in the interval between the shorter and longer follow-up periods, thus once more emphasizing the importance of the latter in assessing the achievements of breast cancer therapy.

A study of grading and also staging indicates that both methods give comparable results; the latter, however, has the slight advantage of revealing the outcome for the most advanced cases (Stage 4).

TABLE XII.—*Stage and Prognosis. Five-year Survivals (All Treatments).*

Stage.	Cases.	Alive at 5 years.	
		No.	Per cent.
1	156	115	74
2	145	61	42
3	126	45	36
4	39	5	13
Total	466	226	48

TABLE XIII.—*Stage and Prognosis. Ten-year Survivals (All Treatments).*

Stage.	Cases.	Alive at 10 years.	
		No.	Per cent.
1	109	52	48
2	83	20	24
3	86	20	23
4	29	0	0
Total	307	92	30

Relation between histology and stage.

We have already drawn attention to the relationship in breast cancer between the histological architecture of the tumour and its tendency to involve the axillary glands (Table IX). A further opportunity is now present to correlate grading with invasive and metastasizing power for this type of growth.

The distribution of the cases according to the stage which the neoplasm has reached is shown for each histological grade (Table XIV). Exactly half of the Grade I tumours have not yet extended beyond the first stage, whilst about a quarter are advanced, belonging to Stages 3 or 4 taken together. Roughly the reverse occurs for the cases of high malignancy. Intermediate figures are present for the intervening Grade II.

TABLE XIV.—*Distribution of Cases in Each Grade According to Stage.*

Grade.	Percentage of cases in each stage.			Per cent total.
	1.	2.	3 + 4.	
I	50	23	27	100
II	31	34	35	100
III	20	36	44	100

These results merely give additional support to the view already expressed with regard to the relation between histology and axillary involvement, that the higher the grade of mammary carcinoma, the greater the tendency for early spread beyond the breast.

Stage and Grade for Prognosis.

It has been shown under the previous heading that there is little to choose between a method of grading and staging in determining prognosis for mammary carcinoma. The vast majority of workers in this field, however, have argued as to the relative merits of either system. But why should the grade and stage be considered only separately, and in the sense of competing with each other to form the basis of a reliable prognostic guide ? A combination of these features would result in a classification which gives information on not only the type of growth, but also its extent when first seen. Would this not bring about a more accurate grouping of cases than could be produced by either system alone ? Indeed, several authors have taken the histological picture into consideration with the state of the axillary lymph nodes, and the value of this has been confirmed here (Table VII). On the other hand, a survey of the literature appears to indicate that a combined system of grading and more complete staging has yet to be presented.

The possible value of this will now be examined, the results being shown in Table XV and emphasized in Fig. 13. The percentage of five-year survivors is seen to range from as high as 87 per cent for Stage 1, Grade I cases down to as low as 6 per cent for those belonging to Stage 4, Grade III. It is also evident that whilst the prognosis in each stage deteriorates with the rise in histological malignancy, the cases with low grade tumours do not conform to the general trend. The outlook for these cases is better in Stage 2 than it is for the intermediate or high grades in the first stage. Furthermore, it is really remarkable

that the low grade cases in Stage 3 have a better prognosis than either the intermediate or high grades in the second and even the first stages. Only a few cases are present in Stage 4, but even here the results are seen to be in accordance with those in the earlier categories.

TABLE XV.—*Stage, Grade and Prognosis. Five-year Survivals (All Treatments).*

Stage.	Grade.	Cases.	5-year survivals.	
			No.	Per cent.
1	I	70	61	87
	II	58	39	67
	III	28	15	54
2	I	31	22	71
	II	64	26	41
	III	50	13	26
3	I	36	25	69
	II	47	14	30
	III	43	6	14
4	I	2	2	100
	II	20	2	10
	III	17	1	6
	Total	466	226	48

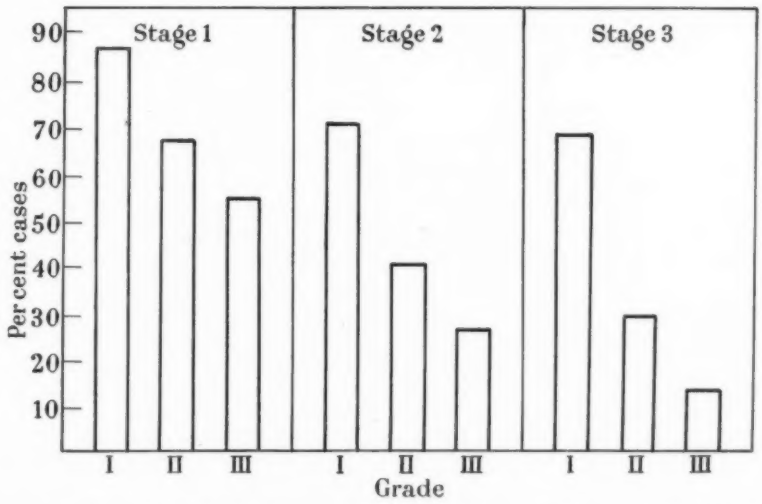

FIG. 13.—Stage, grade and prognosis. Percentage of cases alive at 5 years (all treatments).

From these results the value of linking grading with staging is at once clear. Such a procedure gives a far more accurate indication of prognosis than either system alone. It reveals that all is by no means lost if a patient presents with a Stage 2 or even a State 3 growth provided that it belongs to the lowest grade of malignancy (Grade 1). It also explains why certain cases with Stage 1 tumours give disappointing results, and why some in Stage 3 do surprisingly well.

The ten-year survivals are also given for the 307 cases treated between the years 1936–1937 inclusive (Table XVI, Fig. 14). These are seen to be uniformly less than those for the shorter five-year follow-up, the same pattern of results, however, being maintained.

TABLE XVI.—*Stage, Grade and Prognosis. Ten-year Survivals (All Treatments.)*

Stage.	Grade.	Cases.	10-year survivals.	
			No.	Per cent.
1	I	42	23	55
	II	50	22	44
	III	17	7	41
2	I	14	6	43
	II	37	11	30
	III	32	3	9
3	I	25	12	48
	II	39	7	18
	III	22	1	5
4	I	0	0	0
	II	16	0	0
	III	13	0	0
	Total	307	92	30

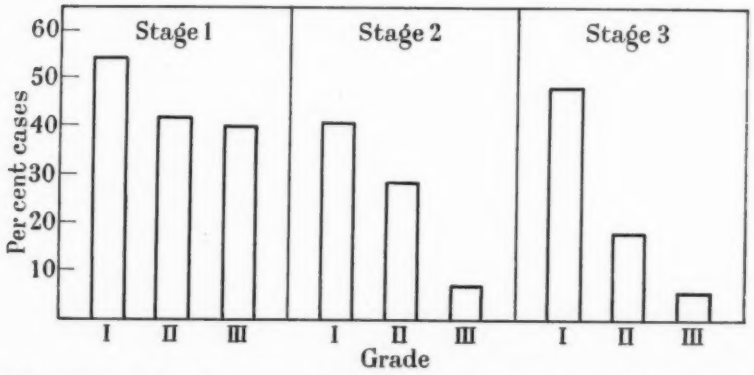

FIG. 14.—Stage, grade and prognosis. Percentage of cases alive at 10 years (all treatments).

Comparison of Two Series of Cases of Breast Cancer.

Having revealed the value of combining histological grading with clinical staging for prognosis in mammary carcinoma, the suggestion is now put forward that the wide range in results produced by different authors for identical methods of treatment depends upon the comparison of incomparable groups of cases. In all probability a similar explanation also accounts for the confusion which exists as to the relative merits of the various lines of therapy advanced for this disease.

The fallacy in grouping cases is considered to result from employing a system of classification based solely upon either the histological or the clinical picture. What evidence is there to support these views ?

With reference to this question, two groups of cases will be taken for study, one treated by surgery alone, and the other by surgery with ancillary radiotherapy. Although it is not the purpose of this paper to discuss the merits of treatment in breast cancer, the results achieved by these two methods will be compared in order to illustrate the discussion.

Method of treatment.

Of the 470 cases in the present series, 209 were treated by surgical measures alone, and 261 by the combined method of surgery and irradiation. These will form the two groups for study. It must be pointed out that the treatment in each group was by no means uniform, there being considerable variation both from the surgical as well as the radiological aspect. In the purely surgical cases, however, the vast majority (180) had a radical mastectomy. In the radio-surgical group there were 206 examples of this operation. Irradiation was predominantly in the form of deep X-rays, either pre-operative, post-operative, or both, a few cases being treated with radium alone or radium and deep X-rays.

The surgical treatment for all but two cases was carried out at the Middlesex Hospital or the Sector Hospitals associated with it during the recent war. The radiotherapy was administered by the Meyerstein Institute of the Middlesex Hospital.

Results of treatment.

According to grade.—Let us first consider the results achieved for each group according to the grade of malignancy (Tables XVII, XVIII). It is evident that the survival rates obtained by surgery alone appear to be superior to those of the combined treatment for all three classes of tumour.

TABLE XVII.—*Grade and Prognosis—Surgical Cases.*

Grade.	Cases.	Alive at 5 years.	
		No.	Per cent.
I	79	67	85
II	85	44	52
III	45	14	31
Total	209	125	60

TABLE XVIII.—*Grade and Prognosis—Radio-surgical Cases.*

Grade.	Cases.	Alive at 5 years.	
		No.	Per cent.
I	62	44	71
II	106	37	35
III	93	21	23
Total	261	102	39

According to stage.—The next step will be to study the results of treatment with regard to the clinical stage instead of the histological grade (Tables XIX, XX). Here again the largely superior results of surgery are obvious. These findings agree closely with those of Harnett (1948) in a larger series of cases (Table XXI).

TABLE XIX.—*Stage and Prognosis—Surgical Cases.*

Stage.	Cases.	Alive at 5 years.	
		No.	Per cent
1	102	77	75
2	57	29	51
3	43	19	44
4	7	0	0
Total	209	125	60

TABLE XX.—*Stage and Prognosis—Radio-surgical Cases.*

Stage.	Cases.	Alive at 5 years.	
		No.	Per cent.
1	54	38	70
2	88	32	36
3	83	26	31
4	32	5	16
Total	257	101	39

TABLE XXI.—*Stage, Treatment and Prognosis* (*Harnett*, 1948).

Stage.	Alive at 5 years.	
	Radical operation. Per cent.	Radical and radiotherapy. Per cent.
1	68	64
2	44	37
3	34	32
4	0	21
Total	48	40

Comparison of Tables XVII and XVIII with Tables XIX and XX reveals comparable results for both grading and staging systems. The latter, however, shows the prognosis for the most advanced cases.

Possible reasons for the inferior results of the radio-surgical cases.

Variation in treatment.—In both series of cases the exact mode of treatment showed much variation. The vast majority of the surgical patients, however, had a radical mastectomy. On the other hand, in the radio-surgical group, although this operation was performed in the majority, there were many instances of more conservative surgery, which included 37 simple mastectomies and 17 local excisions. Because of these facts it may be argued that the variation in treatment produced incomparable groups of cases. Thus the examples of non-radical surgery may, for purposes of comparison, throw an unfair disadvantage on the group receiving ancillary radiotherapy. Any conclusions drawn as to the superiority of surgical over radio-surgical treatment may, therefore, be erroneous.

With this in view, the survivals of a more uniform type of therapy were taken for study, the comparison being made between patients treated by radical surgery alone and radical surgery with irradiation (Tables XXII, XXIII). The results of the former treatment, however, are still seen to be superior to those of the latter. In fact they are almost identical to those given previously for the groups in which the radical operation was not universal (Tables XVII, XVIII). Similar results were obtained when the cases were taken according to stage.

TABLE XXII.—*Results of Radical Mastectomy alone, According to Grade.*

Grade.	Cases.	5-year survivals.	
		No.	Per cent.
I	66	59	89
II	77	40	52
III	37	14	38
Total	180	113	63

TABLE XXIII.—*Results of Radical Mastectomy and Radiotherapy, According to Grade.*

Grade.	Cases.	5-year survivals.	
		No.	Per cent.
I	31	21	68
II	78	27	35
III	60	15	25
Total	169	63	37

The greater achievement of surgery alone over the combined attack is again seen in Harnett's (1948) figures which are also for the radical operation and are classified by stages (Table XXI).

From these facts it is clear that the variation in treatment by surgery in the present material does not appear to interfere with the fair comparison of the

surgical and radio-surgical groups of cases, and cannot be called upon to account for the inferior results of the latter.

Distribution of cases.—The application of either a grading or a staging system separately for the classification of patients with breast cancer may result in fallacious grouping.

Let us first of all consider a system of grading. In this instance a false impression may be given when the results of two series of cases are compared owing to the possible unequal distribution of clinical stages in corresponding histological grades. Thus, in any given grade one of the series may have more early and fewer advanced growths than the other. In fact this has been found to be the case in the present investigation.

Reference to Table XXIV and Fig. 15 shows the distribution of the early and later stages in the three grades of malignancy according to treatment. To simplify the picture Stage 2 has been omitted from the histogram and Stages 3 and 4 taken together. It is evident that in each class of tumour there are more early and fewer advanced cases in the series treated by surgery alone than by the combined method.

TABLE XXIV.—*Distribution of Cases in Each Grade According to Stage and Treatment.*

Grade.	Stage.	Surgical cases.		Radio-surgical cases.	
		No.	Per cent.	No.	Per cent.
I	1	50	63	20	33
	2	16	21	15	25
	3	13	16	23	39
	4	0	0	2	3
Total		79	100	60	100
II	1	36	42	22	21
	2	28	33	36	35
	3	18	21	29	28
	4	3	4	17	16
Total		85	100	104	100
III	1	16	36	12	13
	2	13	29	37	40
	3	12	26	31	33
	4	4	9	13	14
Total		45	100	93	100

Total cases, 466

Our next step is to consider staging. What of the distribution of histological grades of malignancy in the various clinical stages? Reference to Table XXV and Fig. 16 shows a similar arrangement to the above. For the sake of simplicity

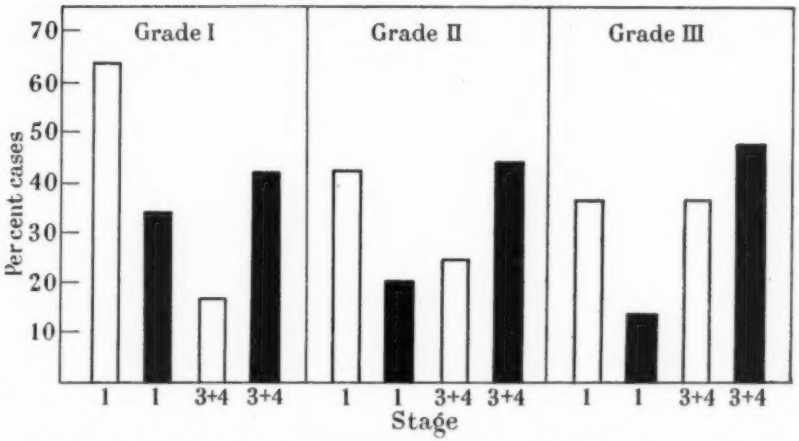

FIG. 15.—Distribution of early and advanced cases according to grade and treatment.
□ Surgery cases. ■ Surgery—radiotherapy cases.

only Grades I and III are shown in Fig. 16. Stage 4 is omitted owing to the small number of cases subjected to surgery alone in this group. Here there are seen to be more low and fewer high grade tumours in each stage of the surgical as compared with the radio-surgical cases. Furthermore, of all the neoplasms of low grade malignancy (Grade I) 57 per cent were in this surgical group in contrast to only 30 per cent of the highly malignant type (Grade III) (Table XXVI). Hence, as with grading, so staging should not be employed as the sole factor in comparing the results of different treatments.

It is well known that the more advanced cases are referred for radiotherapy, and this accounts for the distribution shown in Fig. 15. What has not been previously clear is that the cases in a corresponding stage for both treatments may not be strictly comparable. In the present series more are of high and fewer of low grade malignancy in the group receiving radiotherapy (Fig. 16). The clinician is unaware of the histology of the tumour he examines before considering treatment. What, then, may account for this fact ? Is it merely fortuitous ? Chance, it is true, may play a part, but this is not the only possibility.

Although two groups of patients are placed in the same clinical stage, there may be some difference in the extent of the growth for which no allowance is made in our present staging systems. For example, the number of glands involved in Stage 2 and the degree and extent of attachment in the third stage are variable features. The surgeon is probably influenced by such matters to send the more advanced cases of any particular stage for ancillary radiotherapy. In view of the parallel revealed between histological picture and extent of tumour (Tables IX, XIV), it is these unfavourable cases which also tend to be of high grade malignancy, hence the distribution in Fig. 16.

Here we have investigated two series of cases, each subjected to a different line of therapy. The same problems of classification, nevertheless, are applicable to cases treated by identical methods. For example, in the corresponding stages

TABLE XXV.—*Distribution of Cases in Each Stage According to Grade and Treatment.*

Stage.	Grade.	Surgical cases. No.	Surgical cases. Per cent.	Radio-surgical cases. No.	Radio-surgical cases. Per cent.
1	I	50	49	20	37
	II	36	35	22	41
	III	16	16	12	22
Total		102	100	54	100
2	I	16	28	15	17
	II	28	49	36	41
	III	13	23	37	42
Total		57	100	88	100
3	I	13	30	23	28
	II	18	42	29	35
	III	12	28	31	37
Total		43	100	83	100
4	I	0	0	2	6
	II	3	43	17	53
	III	4	57	13	41
Total		7	100	32	100

Total cases 466.

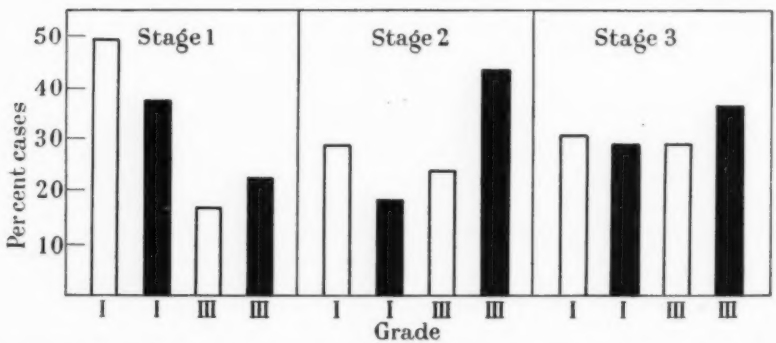

FIG. 16.—Distribution of cases of low and high grade malignancy according to stage and treatment.
□ Surgery cases. ■ Surgery—radiotherapy cases.

TABLE XXVI.—*Distribution of Cases According to Grade and Treatment.*

Grade.	Surgical series. Per cent.	Radio-surgical series. Per cent.	Total. Per cent.
I	57	43	100
II	45	55	100
III	30	70	100

of two or more groups of cases, there may be a widely differing number of tumours of high grade malignancy. It is, therefore, useless to compare results obtained by different authors if the cases are taken according to stage and no allowance is made for type of growth. In this instance chance is probably the chief factor accounting for the distribution of the tumours. A process of selection, however, cannot be ruled out, individual surgeons having, to some extent, personal criteria for deciding on the measures to be adopted for a given case.

Neglected routes of extension.—Reference will be made to avenues of extension of breast cancer which are largely neglected in both prognosis and also treatment. These are via the internal mammary chain and the supra-clavicular glands. It is likely that the tumours of high grade malignancy tend to spread early via such pathways in the same way as they involve the axillary lymph nodes. Of these unfavourable Grade III cases nearly 70 per cent (Table XXVI) were referred for irradiation, thus emphasizing the disadvantage placed on the radiotherapy group when it comes to the comparison of treatment results.

To sum up : It is postulated that in a given stage certain cases have unfavourable clinical features, which influence the surgeon to refer them for radiotherapy. The tumours of these patients tend to be of high grade histological malignancy, and have probably also extended via routes, as yet, not given adequate consideration in prognosis and treatment. This may account, at least to some extent, for the unfavourable results achieved here and elsewhere (Adair, 1943 ; Truscott, 1947 ; Harnett, 1948) by ancillary radiotherapy. Careful selection of cases is, therefore, probably responsible for the superior results of surgical measures alone.

On the other hand, a number of authors report better results for the combined treatment. In these instances the achievement of radiotherapy is probably even greater than appears on first examination owing to the unfavourable features which tend to be present in cases referred for this treatment. Alternatively the arrangement of the cases may be more comparable than in the present investigation.

It has been pointed out that the problem of grouping also applies to series treated by identical methods, though here chance rather than selection is probably a more important factor accounting for the distribution of the patients.

Stage, grade, treatment and prognosis.

By linking staging with grading a more accurate system for determining prognosis has been introduced. With this method it is hoped that further light may be thrown on some of the problems of breast cancer, and possibly the true merits of the various treatments of the disease assessed.

When this clinico-pathological system is applied here, will the results achieved by ancillary radiotherapy appear to be any better ? This question, unfortunately,

cannot be answered for the present because the cases available for such a study were too few in number. Thus the 209 surgical and the 261 radio-surgical patients were each divided into twelve groups (four clinical stages, each containing three histological grades). Consequently the numbers in each were too small to be significant for purposes of comparison. Further work is under way to collect a larger series to enable this to be undertaken.

Treatment and Prognosis.

A paper on prognosis in mammary carcinoma cannot be complete without reference to the effect of treatment. As already pointed out, it is not our intention to undertake a detailed study at this time into the merits of the different modes of therapy. This problem, which requires a larger series of cases than is at present available, will form the subject of a separate inquiry. Suffice here to indicate some of the points to be investigated.

First of all, does treatment prolong life ? Several authors have published figures on the natural duration of untreated breast cancer. The five-year survival rate appears to be about 20 per cent. Harries (personal communication), at the Middlesex Hospital, quotes 19 per cent for the five-year follow-up and 4 per cent for a period of ten years among some 220 cases. These figures are inferior to those obtained by treatment, which is universally accepted as being of definite value in prolonging life, except perhaps in the very advanced cases.

The next problem which arises is whether the type of treatment influences the outlook. It is here that the controversy begins. How radical should surgery be ? Of what value is ancillary radiotherapy ? Such questions as these are still far from being settled. We have shown that in all probability the dispute arises from comparing groups of cases which are not strictly comparable.

Once the cases are classified more accurately, such as on the lines indicated earlier in this paper, and only then, shall we be in a position to study the merits of various treatments. We may find, for example, that less radical surgery may be adequate for the control of certain cases such as those with Grade I, Stage 1 tumours, whilst radiotherapy may be an important ancillary measure for some cases, but on the other hand of little use for others.

From time to time authors have claimed good results for less radical surgical procedures in breast cancer. Grace (1937) in America obtained a 56 per cent five-year survival rate among 40 cases treated by simple mastectomy and radiotherapy. This author considers that " the cellular structure of the tumour is the dominant factor in prognosis, and surgical technique, irrespective of its radicalism, plays a definite secondary role." Some support for this view is perhaps to be found in the paragraphs which follow.

In the present series 37 cases were treated by simple mastectomy and radiotherapy, and 41 per cent were alive at the end of five years. This agrees with Harnett's figure of 40 per cent in a group of 100 similar cases (Harnett, 1948).

Good results have even been claimed for local excision. This operation, followed by irradiation, was performed in 17 patients of the present investigation, and 65 per cent survived five years. Here again agreement is found with Harnett (1948). A group of 45 cases in his series were treated by local excision with implantation of radium, and 63 per cent were alive after a similar period of time.

Among a large number of cases reviewed by Adair (1943), there were 98 examples of the simple operation and 72 of local excision, the majority being given ancillary irradiation. The survival rate in the former group was 62 per cent, and in the latter 67 per cent.

What do these results mean? Can it be possible that more conservative surgical measures than the radical operation are sufficient for the control of mammary cancer? Such, indeed, is the view in this country of Keynes (1937), and more recently McWhirter (1948*a*, 1948*b*).

We have already suggested that when cases are properly grouped according to both stage and grade it may be found that less radical surgery may be adequate for certain types of case. Thus, of the 17 cases in the present series which were treated by local excision and radiotherapy with a survival rate of 65 per cent, 12 belonged to Stage 1, and 10 of these had Grade 1 growths. Of these 10, 90 per cent were alive at the end of five years. The good results in this group probably depend upon the early stage and low malignancy of the tumours.

We conclude this section by emphasizing that the treatment most likely to achieve success in the control of breast cancer cannot be assessed at the present time. This will become possible only when cases are classified more accurately, such as on the clinico-pathological basis indicated here.

Fallacies in Grading and Staging.

We have advocated a system of classifying breast cancer based upon the grade and stage of the growth. Let us now examine these two features for any possible fallacies.

Grading.

Histology of primary growth.—The most frequent objection to grading as an index to prognosis in mammary carcinoma is that sections cut from different parts of the same tumour may show marked histological variation. This may be true in some cases, but its importance has been over-emphasized. In spite of variability a definite microscopic pattern can be recognized for the vast majority of neoplasms of this type. It is not even necessary to have numerous sections to appreciate this picture; one or two portions of tissue of reasonable size taken from the periphery of the growth are adequate. However, in those cases where there is much variability in appearance, the most malignant part of the section is considered.

To test these views the following simple investigation was undertaken: Two sections were cut from different parts of the periphery of each of 25 consecutive breast cancers. The 50 slides thus obtained were labelled with a code number and then mixed. Grading was performed, the code of the accompanying twin to each section being unknown to the examiner. It was found that the pair of sections of each breast had been placed in the same grade of malignancy.

Two further points on the histology of the primary neoplasm require attention. Firstly, grading of cancer in minute fragments of tissue is unreliable, and should not be attempted. Secondly, frozen sections are difficult to assess, and where possible, grading should be confined to tumours prepared by the paraffin technique.

Histology of metastases.—Should the degree of histological malignancy of the metastases vary considerably from the primary tumour, a false impression as to

prognosis may be gained if only the growth in the breast is studied. For example, a tumour of low malignancy may give rise to metastases in which a change of biological character takes place. If the deposits become more malignant then the prognosis will probably be altered. In point of fact, there is reason to believe that an alteration to greater malignancy occurs only on very rare occasions. Thus, Patey and Scarff (1929), in a series of 110 cases, found that the metatases in the axillary nodes were of the same grade as the primary in 82 per cent, of a lower grade in 16 per cent, and of a higher grade in only 2 per cent. Since a considerable proportion of cases tend to show axillary deposits of lower malignancy than the primary growth, grading should be confined to the latter.

Staging.

Axillary glands.—The importance of considering the extent of the growth in grading has already been pointed out. The state of the axillary glands is usually taken as the arbitrary standard for determining whether the growth is confined to the breast or not. This feature is usually assessed clinically in a staging system. It is unsafe, however, to rely on this form of examination for glandular involvement. Non-palpable glands may show histological invasion, and conversely, palpable glands may exhibit only inflammatory changes. A recent statistical survey of breast cancer (Harnett, 1948) revealed 39 per cent of clinically free glands to be invaded microscopically. In 19 per cent of cases with clinical glandular involvement the enlargement proved to be non-malignant. Comparable figures to these are also given by other writers, such as Harrington (1935), Lachman (1944), Riddell (1948) and Ledlie (1948). Greater accuracy in prognosis will therefore be achieved if glandular invasion is determined, where possible, histologically. This has been the rule in the present series.

Saphir and Amromin (1948), on the other hand, have shown that even routine histological examination of the axillary nodes is open to error. By serial sections they discovered involvement of these structures in 33 per cent of a series of cases previously reported as free from metastases.

Internal mammary glands.—Other routes of spread for breast cancer have been largely neglected both in prognosis and treatment. One of the chief pathways is via the internal mammary chain of glands. Handley (1922, 1927) was the first to stress the importance of this avenue of extension, and advocated implantation of radium in the intercostal spaces at the time of operation. Little attention appears to have been given to this problem until Scarff and Handley (1938) suggested that disappointing results in Stage I cases may be explained by this mode of spread. It was left, however, to Handley and Thackray (1947, 1949) to provide histological evidence on this point. These authors have shown that extension via the internal mammary nodes may occur early and not infrequently in the absence of axillary metastases. This applies particularly to growths situated in the inner half of the breast ; of 17 such cases 12 had deposits in the internal mammary chain, in 2 of which the axilla was not also involved. In spite of this, Truscott (1947) and Harnett (1948) were unable to show that the site of the growth plays an important part in prognosis.

Supraclavicular glands.—A further mode of spread which has not received adequate consideration is via the supraclavicular glands. Halsted, as long ago as 1892, was the first to practise removal of these structures in the radical treat-

ment of cancer of the breast (Rodman, 1904). Twelve years later Rodman (1904) advocated this procedure in those instances where the glands are palpably enlarged, or the topmost axillary nodes show macroscopic involvement. This problem appears to have been subsequently largely neglected. Recently, however, interest has been revived by the work of Andreassen and Dahl-Iversen (1949). These authors removed the supraclavicular nodes as part of the radical mastectomy, and found microscopic involvement in 17 per cent of cases. With invasion of the axilla this figure increased to 33 per cent. If the axilla was free from growth, the supraclavicular nodes did not appear to be invaded. The writers concluded that dissection of the supraclavicular region should be performed if there is extension to the axilla.

In those cases where the axilla appears to be free from involvement at the time of operation and the prognosis subsequently turns out to be poor, invasion has probably occurred by routes such as the internal mammary chain, and perhaps the supraclavicular glands. An attempt to assess the state of the latter is made clinically, but involvement may be microscopic and these structures are not removed as a routine. The state of the internal mammary nodes is usually entirely neglected. These channels of extension may account for at least some of the 13 per cent mortality for Stage 1, Grade I cases (Table XV).

After considering these facts it would appear, as Handley and Thackray (1949) point out, that there are but few growths which are really confined to the breast. It is therefore perhaps surprising to find such good results for even what appear to be early tumours of low malignancy. In the present investigation 94 per cent of Grade I cases without axillary node involvement were alive at five years (Table VII).

CONCLUSIONS.

A classification for patients with breast cancer which depends upon either a system of staging or grading tends to group cases that are not strictly similar. Comparative studies of such groups is, therefore, apt to be misleading. Unfortunately, methods based upon staging alone are in general use at the present time, and as long as they continue to be employed, so it is likely that there will be no accurate assessment of the various lines of treatment.

Several attempts have been made in the past to establish a more reliable prognostic system, and consequently a better classification, for cases of mammary carcinoma. Chief success, from the point of accuracy and simplicity, appears to have been from histological grading, on the lines indicated by Patey and Scarff (1928), combined with the state of the axillary glands. The value of this method has been confirmed here.

A still more useful method of determining prognosis by linking staging and grading has been presented. Wider adoption of this plan may clarify the position regarding the relative merits of the various treatments advanced for breast cancer. It may even be possible to assess the mode of therapy most likely to achieve the best results for certain groups of cases.

There are two reasons why grading is of importance in prognosis. Firstly, the histological appearance of the tumour is closely related to its " potential malignancy " (i.e. the tendency to spread and form metastases). Secondly, it provides some measure of the probable extent of the growth when the patient is first seen, the most malignant tumours having spread furthest. This latter feature com-

pensates to some degree for those pathways of invasion which are not adequately considered in our present staging systems.

From what has been said, it is evident that the morbid histologist can contribute important information towards the problems of mammary carcinoma. It would appear, however, that this fact has been largely neglected by those concerned with its treatment. Why has there always been at least a 25 per cent five-year mortality for the most favourable cases, i.e. Stage 1 ? This question has defeated explanation in the past, and will probably continue to do so until such time as the pathologist plays a part in establishing a prognostic system of the type indicated in this paper.

Finally, a plea is made for more attention to histological grading in carcinoma of the breast, and a closer co-operation between clinician and pathologist. It may well be that grading of cancer in other sites, on the principles employed here, will be found of similar value.

SUMMARY.

The controversy which exists as to the relative merits of the different methods of treatment for carcinoma of the breast has led to the present investigation, the prime object of which has been to establish a more accurate classification for this disease.

Previous attempts at histological grading in breast cancer have been reviewed.

Grading has been studied in a series of 470 cases of mammary carcinoma. It has been accomplished without technical difficulty, and a close parallel was found between the histological picture and the period of survival.

The results of grading have been compared with those of clinical staging. Both methods, when employed separately, give comparable results.

The grade of the tumour taken together with the state of the axillary lymph nodes gives a more accurate indication of outcome than either grading or staging alone. For example, 94 per cent of Grade I cases without glandular involvement were alive at five years compared with 16 per cent of Grade III with this complication.

A clinico-pathological system for determining prognosis by linking stage and grade has been presented. This procedure casts light on some of the hitherto unexplained clinical problems of breast cancer. It offers an explanation for disasters in early cases, and also for remarkably good results obtained in advanced stages. In indicates that all is not necessarily lost if a patient presents with a Stage 2 or even a Stage 3 growth, provided it is of low grade histological malignancy.

The five-year results achieved by surgical treatment both alone and in association with radiotherapy have been presented. Inferior results were obtained for the combined attack.

It has been shown that in a given clinical stage or histological grade the cases treated by surgery alone are not strictly comparable to those subjected to surgery and radiotherapy. The latter were found to have additional unfavourable features. This fact has been invoked to explain the disappointing results of ancillary radiotherapy, case selection probably accounting for the superior results of surgical treatment alone.

A classification of breast cancer based upon either a system of grading or staging separately brings about a grouping of cases which are not strictly

similar in type. The comparison of such atypical groups may well be responsible for the wide variation in results obtained by different workers for identical methods of treatment. In addition this has probably also played a major part in bringing about the present-day confusion as to the relative merits of the various measures advanced for the control of the disease.

Attention has been drawn to the possible prognostic and therapeutic importance of lymphatic spread to other glands than those in the axilla.

Possible fallacies in grading and staging have been discussed.

Brief reference has been made to the effects of treatment on prognosis. The good results of conservative surgical procedures have been noted.

Note by T. E. Cowan, Esq., F.C.I.S., F.R.S.S.: The interpretations arrived at from the tables shown are statistically sound and call for no special comment.

I am indebted to Professor R. W. Scarff for introducing me to his system of histological grading of breast cancer, and for help and encouragement; to Dr. A. C. Thackray for kind advice and criticism; to Dr. D. K. Sambrook for assessing the clinical stage of the cases; to Miss Chambers of the Follow-up Department for tracing the patients, and to the Photographic Department for assistance with the microphotographs.

For the cases employed in this work my gratitude is due to the surgeons of the Middlesex Hospital and War-time Sector Units, and to Professor B. W. Windeyer, of the Meyerstein Institute of Radiotherapy.

The expenses of this investigation were defrayed by the British Empire Cancer Campaign.

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THE TECHNIQUE OF RADICAL MASTECTOMY: WITH SPECIAL REFERENCE TO THE MANAGEMENT OF THE SKIN SHORT CASE AND THE PREVENTION OF FUNCTIONAL DISABILITY.

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Received for publication May 19, 1950.

MORBIDITY.

Paradoxically to-day the morbidity is more worrying than the mortality following the operation of radical mastectomy. It would be wrong to give the impression that undesirable sequelae are common, yet such complications as wound sepsis, necrosis of the skin edges and a poor functional result are still seen with greater frequency than one would expect following an operation which has been abundantly practised by innumerable surgeons all over the world for over half a century. These particular sequelae are essentially a reflection on the judgment or the executive skill of the operator and not upon the operation itself, because with care they need not happen. Furthermore, such indifferent results become known, and because they lose nothing in the telling may have a serious deterrent effect upon patients contemplating advice about a newly acquired lump in the breast. It is to be remembered that we are operating under almost ideal conditions—the patients are constitutionally well and often near the prime of life, the anatomy of the area is normal and not distorted as it might be, and there are none of the embarrassments associated with operations within a body cavity. A very high degree of technical excellence should therefore be our aim, both in regard to the thoroughness of the dissection which has as its objective the extirpation of the disease, and in regard to the care with which this is carried out, which can so much determine the degree of ultimate functional disability.

THE MANAGEMENT OF THE "SKIN SHORT" CASE.

The "skin short" case is one in which it is judged that it will not be possible to close the wound completely following mastectomy.

A shortage of skin is most commonly encountered in women (*a*) with small breasts or (*b*) with large tumours.

There are three features of the "skin short" case which need some explanation. I will call them (i) anticipating the gap, (ii) siting the gap, (iii) covering the gap.

Anticipating the Gap.

It is of prime importance to recognize a shortage of skin at the first clinical examination. The early discovery of a shortage has valuable practical bearings in two directions: First, the actual sequence of treatment itself—irradiation or

surgery—may be influenced ; second, the surgeon is forewarned, and so can avoid being surprised into an awkward situation at the end of a long operation.

Pre-operative irradiation in the "skin short" case.

An assessment—an estimate—should be made in all new cases of cancer of the breast of the amount of skin that will be left for closing the wound after the breast and tumour have been removed. It will be found that patients fall into two categories :

(1) A larger group in whom it is judged that it will be possible to approximate the skin edges in the usual way without tension.

(2) A smaller group in whom primary removal of the breast would leave and should leave a gap between the skin edges (Fig. 1). In this smaller group we are faced with something of a dilemma, and this applies equally to simple mastectomies. If we remove too little skin, secondary nodules will appear for certain and quickly ; if we remove a more generous amount—as we must—we shall have to be prepared to deal with a gap. In these patients two alternatives are open to us : either we can proceed to radical mastectomy, skin graft the resulting gap, and hope that the skin covering it will heal in a reasonable time and so allow early post-operative irradiation, or we can anticipate the probable delay in healing by giving the X-ray treatment before the operation.

Personally in this small group I prefer to give the X-ray treatment first, because in practice unfortunately every skin graft does not take uniformly, so that a proportion of cases are left to heal—at least in part—by granulation, and this takes time ; indeed, sometimes so much time that post-operative X-ray treatment may be delayed to a point where its usefulness is open to question. For this reason it is suggested that if by careful pre-operative clinical assessment we can anticipate and so forecast such a defect arising, we should consider giving the X-ray treatment before the operation. The use of pre-operative irradiation does not in any way absolve the operator subsequently from still adhering to the principle of excising a wide area clear of the growth. The argument is, that once the course of therapy has been given, delay in healing of the wound has not the

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EXPLANATION OF PLATES.

FIG. 1.—"Skin short" case : A "skin short" case with full range of movement at the shoulder post-operatively. This patient was treated by pre-operative irradiation followed by radical mastectomy with an immediate skin-graft. The satisfactory functional result can be attributed to (i) adoption of the "arm raised" position before approximation of the skin flaps, (ii) the early recognition of the inevitability of "a gap" in this type of case and of the need for a skin-graft.

FIG. 2.—Full range of movement : The "arm raised" position is the most delicate test of function at the shoulder following mastectomy ; six months should be allowed after the termination of all treatment for the skin to regain its suppleness before assessing function ; inability to raise the arm vertically after such an interval must be regarded as an imperfect functional result.

FIG. 3.—Marginal flap necrosis : The illustration shows an area of skin loss as a result of damage to the blood supply of the lateral flap during its elevation. The dead portion should be excised (dotted line) and skin grafted.

FIG. 5.—Functional disability : Restricted movement at the shoulder following mastectomy from closure of the wound with the arm to the side (skin displacement). The other common cause of restricted movement is marginal necrosis with healing by granulation and scar tissue (skin loss).

FIG. 7.—Nerve to M. latissimus dorsi : The nerve to latissimus dorsi (left) has been divided without effect upon the patient's muscular power at the shoulder.

Riddell.

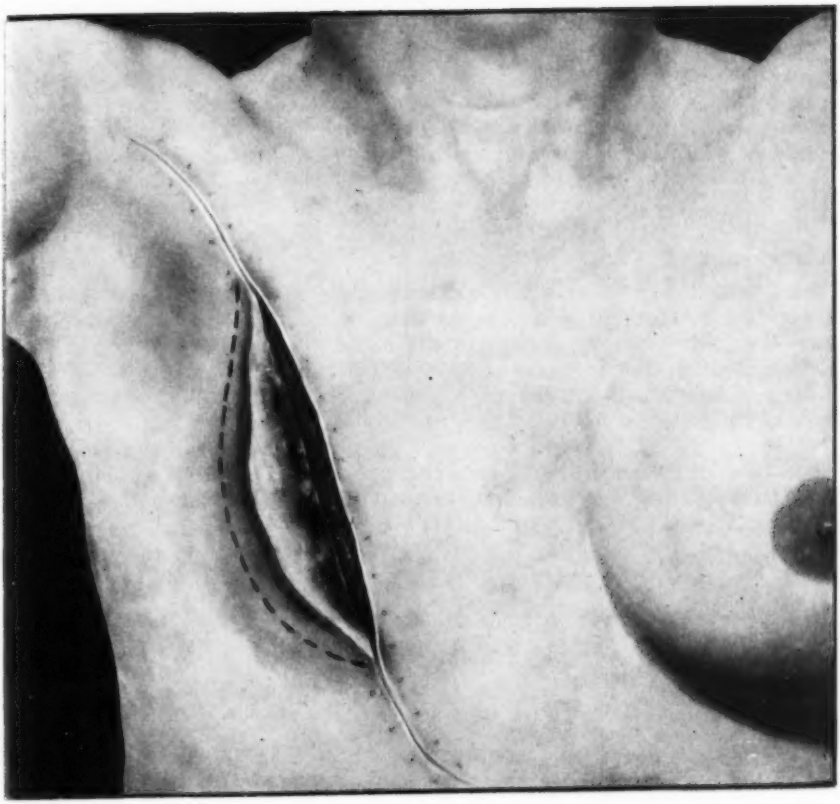

Fig. 3.

same significance, as it holds up nothing except the ordinary convalescence of the patient.

Siting the Gap.

The gap should never be sited in the axilla, but always as far down the chest wall as possible.

Covering the Gap—Skin-grafting.

The question often arises as to whether we should skin-graft a gap or leave it to heal by granulation. The answer must be that healing is much accelerated by skin-grafting. At the same time it is not vital to graft; what is vital is that a gap should be left in a skin short case; whether a dressing of skin is put on that gap or not is almost a secondary consideration, although skin is very clearly to be preferred to scar tissue if the gap is a large one.

The time to graft.

The decision as to whether the graft is applied at the time of the mastectomy or not depends solely on the condition of the patient at the end of the operation. If this is unsatisfactory, as it may be in an elderly patient, no harm is done by leaving a raw area and grafting early in convalescence, usually at the time of the first major dressing. If, however, the patient's condition is satisfactory, grafting of the gap should be done there and then. If, in addition, we have made our pre-operative reconnaissance, the procedure is robbed of its only disagreeable feature, the element of surprise, since a donor area will have been chosen and prepared and the few special instruments and dressings will be immediately available.

THE PREVENTION OF FUNCTIONAL DISABILITY.

Functional disability following radical mastectomy should be rare (Fig. 2). It is due to *skin loss*. There are two quite different ways in which this can come about.

Destruction of skin.

In the first the skin loss is due to damage to the blood supply during its elevation. This accident most commonly occurs as the dissection approaches the anterior border of latissimus dorsi, which corresponds with the deepest and darkest part of the wound; it is usually due to hurrying this part of the reflection in a field obscured by haemorrhage; the blood supply may also be cut off from a portion of the flap by the use of tension sutures and with the same result. Both are surgical injuries. Both result in varying marginal skin loss.

As soon as it becomes clear that an area of skin has been deprived of its blood supply no time should be lost in excision of the dead portion under a short general anaesthetic (Fig. 3). Such prompt action will greatly shorten the delay in healing. To wait for the devitalized area to slough off of its own accord is to prolong convalescence by many weeks. The denuded area should be skin-grafted as soon as the local bacteriology offers a reasonable chance of a "take."

Displacement of skin.

In the second category the skin is not lost in the sense that it is destroyed, but is lost by displacement, because skin is borrowed from the axilla and upper arm to close the axillary part of the wound. This is what happens: The wound is being sewn up, and the edges become increasingly difficult to approximate the further we get from the axillary end. At this stage we remember that if the arm is lowered tension will be released (Fig. 4). Most of us on some occasion have

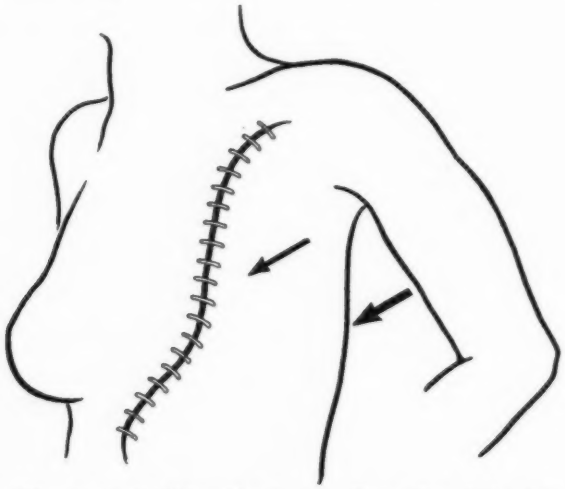

FIG. 4.—The arm to the side.—the wrong position before closing the wound.

been guilty of saying, "Oh, it will come together when we bring the arm down to the side." This should never be done. To yield to this temptation may at the time enable us to close the wound throughout its length and so pass over a temporary difficulty, but is no real solution, as it leaves the patient with the foundation of a permanent deformity (Fig. 5).

The procedure I recommend may be paraphrased—"Never a borrower but a lender be." It is briefly as follows. Before sewing up the flaps at the end of the operation observe two rules (Fig. 6):

(1) Elevate the arm straight above the head; in this position the upper limb makes its maximum demand upon the skin of the axilla; that is to say, this position gives the measure of the exact amount of skin required in the axilla to allow full and unrestricted movements at the shoulder post-operatively.

(2) Slide the skin of the lateral flap well up into the axilla; by making use of the longer contour of the lateral flap in this way an adequate supply of skin in the axilla is assured. In suturing the flaps together exactly opposite points on the two flaps must not be approximated—a slightly higher point on the medial flap will be sewn to a slightly lower point on the lateral flap.

In other words, lend skin from the lateral flap to the axilla; do not borrow it from the axilla and upper arm by bringing the arm down to a right angle or less.

This sliding manoeuvre may result in a gap between the flaps somewhere lower down on the chest wall, but this is a small price to pay for ensuring full range of movements at the shoulder.

I should perhaps add that in my experience functional disability at the shoulder is not related to loss of the pectoral muscles; indeed, it can be quite severe following a local mastectomy, and is not related to failure to employ early

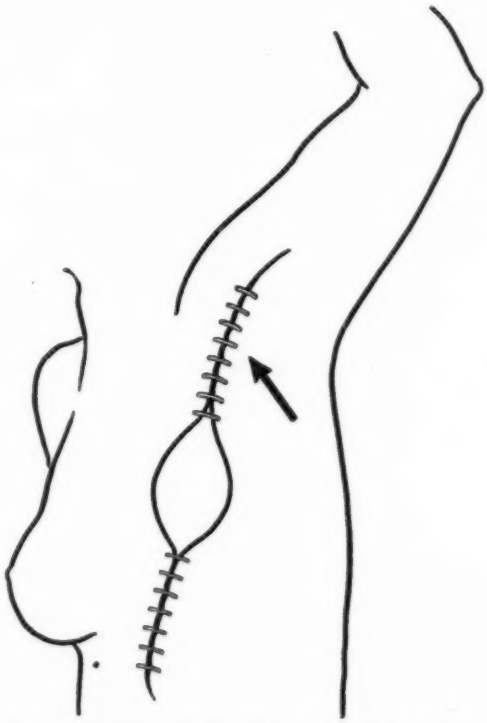

FIG. 6.—The arm raised. The drawing shows the correct position of the upper limb before closing the wound; the skin of the lateral flap should be pushed well up into the axilla before suturing (arrow).

movements of the arm post-operatively, nor is it related to division of the nerve to latissimus dorsi. In a series of cases examined in which the subscapular nerve had been divided, no functional disability could be found by comparison with control cases and with the opposite side, although, of course, there was a positive R.D. present, and none of the patients examined by us complained of any limitation of movement of the arm on the affected side (Fig. 7). A badly placed incision which heals as a band across the axilla does not help, but if there is plenty of axillary skin, such a scar will not of itself seriously interfere with function (Fig. 8).

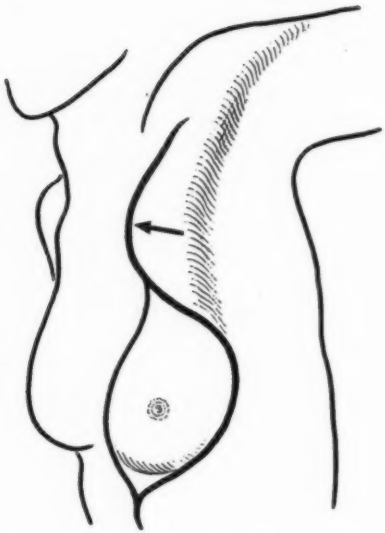

FIG. 8.—Skin incision. The upper or axillary part of the incision should be made well to the medial side of the anterior axillary fold.

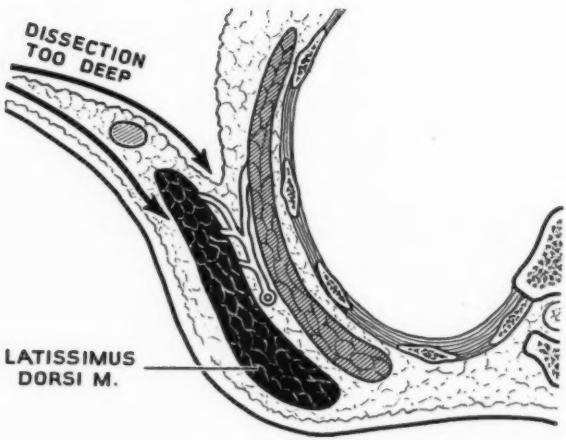

FIG. 9.—Lateral flap. The aim is to display the anterior border of the latissimus dorsi muscle, and in " skin short " cases to mobilize the lateral flap still further by carrying the dissection backwards for two or three inches beyond this point, keeping on a plane superficial to the muscle.

To sum up the steps which should be taken in the prevention of functional disability at the shoulder, I would say approximate the skin edges with the arm fully elevated and never down at the side; if necessary lend skin from the chest wall to the axilla, but never in the opposite direction; excise all dead skin in cases of marginal necrosis as soon as it becomes obvious that it is devitalized and skin-graft the resulting raw area; lastly, discourage all movements at the shoulder joint until the wound is healed, because too early movement may cause separation of the skin edges, and increases the liability to serum collection without any compensating advantages.

TECHNICAL DETAILS.

Elevation of the lateral flap.

In the past insufficient emphasis has been placed on the hazards associated with hurrying the elevation of the lateral flap. Technically this is the only difficult part of an otherwise straightforward operation. Time spent over this stage is time gained later on. The aim is to raise a flap of even thinness, but if by a careless stroke of the knife the flap is cut too thin, the blood supply will be damaged and the whole course of convalescence unnecessarily prolonged. If too thick a flap is raised the dissection may in error proceed on the deep face of latissimus dorsi into the space between this muscle and the serratus anterior, a space which is filled with large veins. There is the additional risk that an invaded gland or glands may be left buried in the fat of the base of a thick lateral flap, where in time its continued growth may produce a so-called axillary recurrence (Fig. 9). True axillary recurrences in experienced hands are rare. Their infrequency is evidence of the effectiveness of the axillary dissection, as it is also a measure of surgical competence in the area.

Blood transfusion.

One word of warning about blood transfusion. If, on admission, the patient is severely anaemic, blood should be given, but let the transfusion be given before the operation; it can be supplemented by plasma or other blood substitute during the operation if necessary. This is a partial contradiction of views expressed earlier in my experience (Riddell, 1948), and is based on the fact that blood transfusion to an unconscious patient carries with it an extra hazard—slight though it may be—for the patient cannot proclaim against the blood if it is incompatible, as would be possible if she were conscious. At the present time blood transfusion is in danger of becoming a routine procedure in certain major operations, regardless of the blood loss expected or encountered.

Details of procedure.

There are details of procedure about which each surgeon has his own ideas (Fig. 10). In my opinion the nurse taking the case should stand at the head of the table and not at the side, as she has a better view of the operation field from this position. The towelling of the operation area should be so arranged, by placing a towel behind the patient's back, that there is no unprotected area on the flank from which the main wound might be contaminated; hot wet packs, which so quickly become cold wet packs and chill the patient, should only be used for haemostatic purposes at the end of the operation; dry hot packs

direct from the baking are better for use during the course of the operation. Drainage should never be employed through any part of the original incision, because it can so easily lead to cross infection of the main length of the wound; it is best carried out through a separate stab incision in the lateral flap, preferably well clear of the hair and sweat-bearing skin of the axilla. Lastly, let us banish for all time the use of tension sutures, and above all the expression

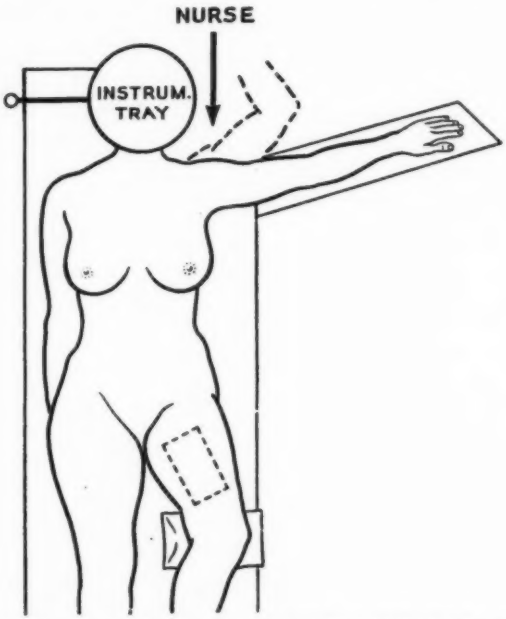

FIG. 10.—The composite drawing illustrates (*a*) the position of the leg for cutting an immediate skin-graft. It is a sound precaution to towel up all "skin-short" cases with the donor area prepared and accessible. (*b*) The position of the arm on the side of operation—outstretched on an arm board with the palm pronated facing the floor. This position is to be preferred to the arm-elevated position (dotted line), which is sometimes followed by a deltoid palsy as a result of the limb falling back by its own weight and so stretching the axillary nerve. (*c*) An instrument tray, conveniently placed at the head of the table. (*d*) The position of the nurse at the head of the table, where she has an unobscured view of the operating field.

"amputation of the breast"—horrible phrase with all it implies of speed, haemorrhage and rough handling.

More and more patients are coming to us in the earliest stages of the disease than ever before. It follows that the potentialities of radical mastectomy were never so high or the quality of its execution so important.

Mortality.

The mortality of the operation is happily low. My own figures, which are not exceptional to-day, are 216 radical mastectomies without operative mor-

tality, that is to say, no patient died within a month of the operation or while still in hospital.

SUMMARY.

It is not in our power to prevent physical disfigurement following mastectomy, but it is in our power to prevent physical disablement. Functional disability usually takes the form of restricted movement at the shoulder and is doubly undesirable, since it not only seriously reduces the range of usefulness of the limb concerned, but aggravates by constant reminder the psychological trauma resulting from the operation. Measures directed towards the prevention of functional disability are discussed.

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THE INFLUENCE OF DIET ON THE WALKER RAT CARCINOMA 256, AND ITS RESPONSE TO X-RADIATION—CYTOLOGICAL AND HISTOLOGICAL INVESTIGATIONS.

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Received for publication July 10, 1950.

IT has been found (Elson and Lamerton, 1949) that the protein content of the diet has a profound influence on the response of the Walker rat carcinoma 256 to X-radiation. Two effects were considered to be concerned in this response: (*a*) the initial inhibition of tumour growth and (*b*) the elimination of the inhibited tumour and cure of the animal. Process (*a*) was found to be favoured by a low protein diet and process (b) by a high protein diet.

Although variations occur within a group of animals maintained on a standard diet of fixed composition, the general type of response to a suitably fractionated X-ray treatment is illustrated in Fig. 1. In animals maintained on the 5 per cent protein diet the tumour often shows an almost immediate growth inhibitory response to radiation treatment. Growth may be nearly stopped for a considerable time, but the tumour usually begins to grow again, at first slowly and then more rapidly, finally causing death. In animals maintained on a 20 per cent protein diet, such an immediate tumour inhibitory response is not usually obtained. The tumour, however, soon reaches a maximum size and then begins to get smaller. The rate at which it decreases in size is at first fairly rapid, but gradually becomes slower, and in many cases the animal is then able to rid itself entirely of the tumour by a " shelling out " process or sometimes by the gradual complete regression of the tumour.

The tumour growth curves (Fig. 1) thus show two rather critical periods, one marked A, where the tumours in the animals maintained on the 20 per cent protein diet reach a maximum size and then begin to regress, and the other B where the tumours of the 20 per cent and 5 per cent protein diet animals are about the same size; but whereas those of the former often continue to become smaller and eventually regress, the latter, after a period of inhibition, usually resume rapid growth, leading to death.

A detailed histological and cytological examination of tumours, particularly under conditions presented by A and B, was therefore undertaken in an attempt to elucidate the part played by diet in influencing the ultimate response of these tumours to radiation.

EXPERIMENTAL TECHNIQUES.

Animals.—Albino rats of both sexes, weighing between 100 g. and 150 g. at the time of tumour implantation were used. They were kept in individual cages, and maintained on the 20 per cent or the 5 per cent protein diet for at least 7

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days before the tumours were implanted. The weight of each rat was recorded at intervals of 2 or 3 days throughout the period of the experiment.

Diets.—The 20 per cent protein diet was the same as that described by Elson and Warren (1947—Table 1) and the 5 per cent protein diet was that described by Elson, Goulden and Warren (1947—Table II). Casein is the main protein constituent.

Tumour measurement.—The growth of tumours was followed by measurements of tumour size with callipers along two axes at right angles. The first measurement was usually made on the 6th day after implantation of the tumour. The estimate of the area (sq. mm.) obtained by multiplying these two measurements was plotted against the number of days after implantation to give growth curves (Elson and Lamerton, 1949).

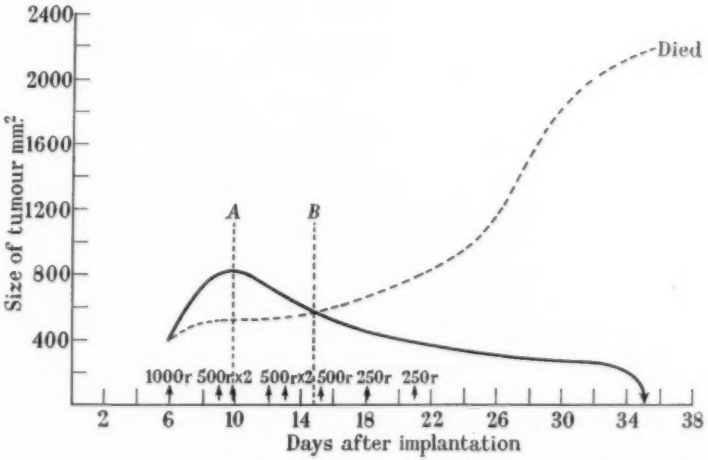


FIG. 1.—Response of the Walker rat carcinoma 256 to X-radiation in animals maintained on high and low protein diets. ——— High (20 per cent) protein diet. - - - - - Low (5 per cent) protein diet.

Radiation technique.—The radiation conditions were those described by Elson and Lamerton (1949). The X-radiation was produced at 140 kVp with a filter of 0·1 mm. Cu giving radiation of half value layer 0·20 mm. Cu. The applicators used were of f.s.d. 15 cm. Under these conditions the dosage rate at the centre of the tumour is about 140 r/min.

Histological technique.—Cross sections of the implanted tumours were made by means of a special cutter with parallel knives giving a section of about 5 mm. thickness. They were fixed in Bouin solution and sections 5μ thick were stained with haematoxylin and orange G.

Cytological technique.—For cytological analysis pieces of tumour tissue were fixed in acetic alcohol (3 parts absolute alcohol, 1 part glacial acetic acid). Squash preparations were made and stained with Feulgen's basic fuchsin.

TABLE I.—*The Various Cell Injuries Induced in the Walker Tumour after* 100 *and* 300 *r.*

I.	II.	III.	IV.	V.		VI.*						VII.†	VIII.‡	IX.	X.	XI.
				No.	%	0.	1.	2.	3.	4.	5+.					
r.	%.															
100	5	6	139	27	19·4	112	20	5	—	—	—	2	0·22	1·1	7.xii.48	13.xii.48
		12	78	23	29·5	55	13	4	1	—	—	5	0·32	9·6	7.xii.48	13.xii.48
		24	50	11	22·0	39	3	—	—	—	—	8	0·06	5·8	12.i.49	19.i.49
		48	50	5	10·0	45	—	1	—	1	—	3	0·12	3·6	12.i.49	20.i.49
		72	50	3	6·0	47	1	—	—	—	—	2	0·02	1·4	12.i.49	21.i.49
	20	6	78	15	19·0	63	7	4	—	—	—	4	0·19	2·0	7.xii.48	13.xii.48
		12	50	18	36·0	32	7	4	2	2	1	2	0·68	8·4	12.i.49	18.i.49
		24	88	14	15·9	74	3	2	—	—	—	9	0·08	4·6	7.xii.48	14.xii.48
		48	50	11	22·0	39	1	1	1	—	—	8	0·12	3·8	12.i.49	20.i.49
		72	50	1	2·0	49	—	—	—	—	—	1	0·0	1·4	26.i.49	5.ii.49
300	5	6	50	29	58·0	21	12	5	3	1	4	4	1·02	8·2	12.i.49	18.i.49
		12	50	33	66·0	17	2	10	6	6	6	3	1·88	14·4	7.xii.48	14.xii.48
		24	50	21	42·0	29	10	—	2	—	3	6	0·62	23·2	12.i.49	19.i.49
		48	50	16	32·0	34	5	2	—	—	—	9	0·18	4·2	26.i.49	4.ii.49
		72	50	9	18·0	41	—	1	—	—	—	8	0·04	5·4	26.i.49	5.ii.49
		96	50	6	12·0	44	—	—	—	—	—	6	0·0	2·0	26.i.49	6.ii.49
	20	6	50	29	58·0	21	15	10	3	1	—	0	0·96	5·0	12.i.49	18.i.49
		12	50	30	60·0	20	9	12	2	2	1	3	1·04	15·2	7.xii.48	14.xii.48
		24	51	22	43·2	29	9	8	2	—	1	2	0·71	19·6	7.xii.48	15.xii.48
		48	50	13	26·0	37	2	1	—	—	—	10	0·08	14·2	12.i.49	21.i.49
		72	50	10	20·0	40	—	1	—	—	—	9	0·04	3·8	12.i.49	21.i.49
		96	50	9	18·0	41	—	—	—	—	—	9	0·0	2·4	26.i.49	6.ii.49

Key to Table I.

I = X-ray dose in roentgens.
II = Protein content of diet.
III = Time after irradiation in hours.
IV = Total number of cells in ana-telophase analysed.
V = Number and percentage of injured cells in ana-telophase.
VI = Number of cells with 0–5 + fragments.
VII = Number of cells with bridges.
VIII = Number of fragments per cell.
IX = Percentage of cells in resting stage with micronuclei (500 cells counted).
X = Date of implantation of tumour.
XI = Date when animal was killed.

* VI : This column includes cells with fragments accompanied by chromosome bridges.
† VII : Number of cells with bridges but without fragments.
‡ VIII : This ratio represents the average number of fragments per cell for all cells analysed.

TABLE II.—*Comparison of Cell Injuries 12 Hours after 100 and 300 r on 5 and 20 per cent Protein Diet obtained in Two Independent Experiments.*

	100 r.				300 r.			
	5%.		20%.		5%.		20%.	
	Exp. I.	Exp. II.	Exp. I.	Exp. II.	Exp. I.	Exp. II.	Exp. I.	Exp. II.
Number of cells at anaphase	75	100	50	100	50	100	50	100
Percentage of cells injured	29·5	19·0	36·0	28·0	66·0	73·0	60·0	80·0
Number of cells with bridges	9	6	5	10	17	28	15	40
Number of fragments per cell	0·32	0·16	0·68	0·26	1·88	0·71	1·04	0·75

Experiment I was carried out and analysed by Dr. F. Devik (December, 1948 to January, 1949). (Full results given in Table I.)

IMPLANTATION AND GROWTH OF TUMOUR GRAFTS.

The conventional method of propagation of the Walker carcinoma consists of the insertion of small pieces of tumour (approximately 7 × 5 × 5 mm.) under the skin of rats. The initial reaction of the host-tissues to the heterologous tumour graft takes place within the first 48 hours. An inflammatory exudate appears around the graft, followed by the development of a primitive connective tissue network, into which tumour cells begin to migrate (Fig. 2). A rapid in-growth of capillaries accompanies the organization of the connective tissue " capsule " and completes the establishment of the subcutaneous graft. Fig. 3 shows an area adjacent to the tumour graft 5 days after implantation. A more detailed description of this process is given by Algire and Chalkley (1945). This phase—which may be referred to as the " take " of tumour—is followed by the second stage. Tumour cells migrate from the graft into the capsule of connective tissue and undergo mitosis ; thus the tumour begins to grow and spread. This process can be clearly observed 4–7 days after implantation (Fig. 4 and 5).

We have observed considerable variation between individual rats in the process of establishment and growth of such tumour grafts. Although factors such as age, sex, weight, etc., and general condition may account for some of this variation, it is probably mainly an expression of genotypic differences present in the colony from which the rats used in these experiments were drawn.

With our present routine for breeding and selection of animals for tumour implantation, when the tumours of a group of ten rats are measured 8 days after implantation the measurements show a distribution of 1 or 2 large tumours, about 5 medium-sized tumours and the rest considerably smaller. That this is largely the result of genetical variation is shown by comparison of the growth rates of tumours in litter mates and by implantation of similar sized pieces of tumour in both flanks of the same animal.

Fig. 6 shows the growth of tumours in rats implanted with similar sized pieces of tumour all derived from the same Walker rat carcinoma 256. The animals were divided into 4 groups, each group consisting of 4 litter mates, 2 male and 2 female ; they were killed 14 days after implantation and the weights of their tumours recorded. It is seen that the 3 most rapidly growing and largest tumours (over 35 g.) all occurred in Group 1, whilst in Group 3 none of the tumours grew well.

That the take and growth of the tumour is largely dependent on the genetical

constitution of the animal and not merely on the nature of the implants or technique of implantation, is also shown very clearly in Fig. 7, which gives the growth curves and weights of tumours implanted in both flanks of the same animal. The growth rates of both tumours were remarkably similar, and if a tumour implant in one flank failed to grow the corresponding tumour in the opposite flank also failed. An investigation into the genetical basis of these differences is clearly desirable, but such an undertaking was beyond the scope of the present

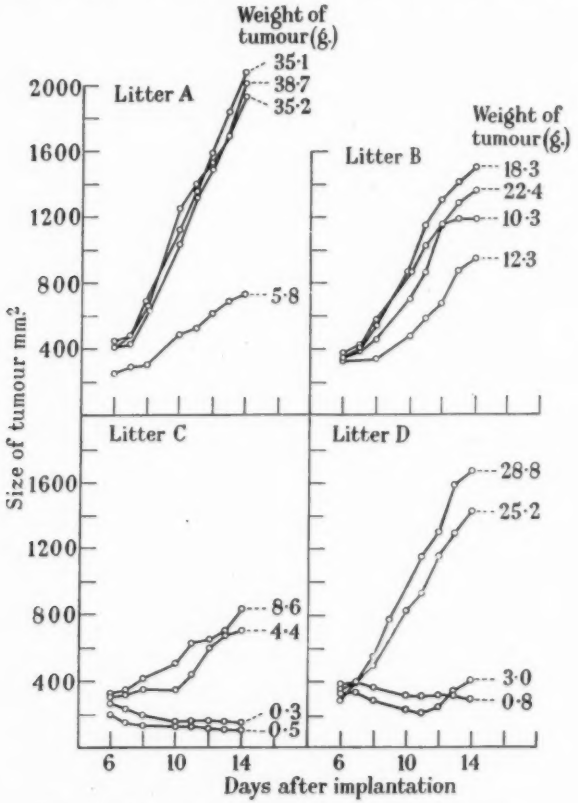


FIG. 6.—Growth of the Walker tumour in litter mates.

study. An attempt has, however, been made to minimize these genotypic differences in the stock rats by selecting only those tumours which had reached approximately the same size (350 to 450 sq. mm.) 6 days after implantation.

Influence of diet on the growth of tumour grafts.

Although there is a very great difference between the growth rate of rats maintained on a 20 per cent protein diet and those maintained on a 5 per cent

protein diet, the difference in growth rate of implanted tumours in animals maintained on these diets is relatively small. The average daily weight increase of rats maintained on our 20 per cent protein diet is about 3 to 4 g., whilst that of those maintained on our 5 per cent protein diet is less than 0·5 g. The mean

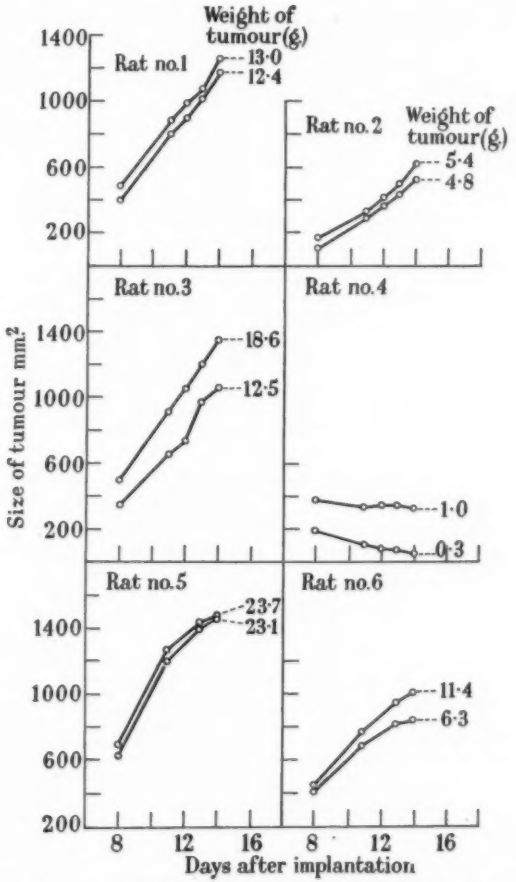


FIG. 7.—Comparison of the growth rate of Walker tumours implanted in the right and left flanks of the same animal.

weight of tumours 14 days after implantation taken from 85 animals maintained on the 20 per cent protein diet was 24 g., whilst that of 86 animals maintained on the 5 per cent protein diet was 20 g. Thus the ratio between the tumour weights on 20 and on 5 per cent protein diet is 1·2, and the average growth rate of tumours is only about 20 per cent higher in animals maintained on high protein than in those receiving a low protein diet.

For comparison of the histology of tumour grafts in animals maintained on high and low protein diets transverse sections were made through the tumour implants and their surrounding tissue 4, 7, 8, 10 and 15 days after implantation. It was found that 4 days after grafting the intensity of the inflammatory reaction as estimated by the number of cells of the reticulo-endothelium accumulated around the graft was greater in rats maintained on the 5 per cent than in those on the 20 per cent protein diet. On the other hand, the capsule of connective tissue was much more distinct in rats on the 20 per cent than on the 5 per cent protein diet. Hence it appears that the low protein diet leads to a greater inflammatory reaction and less organization of the capsule.

These differences are more clearly demonstrated by the behaviour of tumours analysed 7, 8 and 10 days after implantation. The boundary of the tumour is well defined in rats on the 20 per cent protein diet, the migration of tumour cells is negligible and the tumour grows by the very high rate of cell proliferation at the periphery of the tumour parenchyma. On the 5 per cent protein diet, however, there is no definite tumour boundary and many isolated tumour cells can be seen scattered amongst the loose, primitive network of connective tissue, which surrounds the tumour. Included in this " incomplete " capsule are also many small lymphocytes, plasma cells, polymorphs, and macrophages of various types, together with scattered tumour cells, some of which are dividing. During the early stages of the " take " and growth of the implanted tumours the reactions of the surrounding tissues may be very complex and interpretation of the exact course of events is difficult, but in older tumours the histological features of the process are much more clearly defined. The cell-elements of the capsule undergo progressive fibrous differentiation, which can be clearly seen in high protein diet rats with 10 to 15-days-old tumours. The fibrous nature of the capsule is much less obvious in animals maintained on a low protein diet, and its clear demarcation is obscured by the migrating tumour cells. This difference in organization of the capsule on the two diets is shown in Fig. 8, 9.

It should be emphasized that on both diets there may be a considerable variation in the " take " of the graft and in the spread of the implanted tumour in the host, and instances have occasionally been encountered in which the tumour graft took and grew more rapidly in animals maintained on low than in those fed on high protein diet. Such cases were, however, rare, and the effect of the diet is so marked that the differences in capsule-formation and organization between animals maintained on high and low proteins diets can easily be detected in most cases.

Influence of diet on the host reaction to implants of normal rat tissue.

In the study of the reaction of the host rat to the implantation of tumour grafts the fact that the tumour graft is itself growing may very often obscure the observations of the organization of the newly formed connective tissue capsule. It was felt that the introduction into the subcutaneous tissues of a " passive " non-growing heterologous tissue fragment such as muscle instead of the " active " tumour fragment, would make it easier to analyse the influence of environmental factors, etc., on the host reaction.

Pieces of muscle of about the same size as the usual tumour implants, taken from the leg of a second rat were implanted under the skin of the host animal.

In the subsequent reaction to this muscle implant two phases could be clearly distinguished: (*a*) The inflammatory tissue response, (*b*) the formation of a capsule around the implant.

The inflammatory reaction (*a*) is intense 3 days after implantation in rats receiving either the 20 per cent or 5 per cent protein diet, but in those maintained on the 20 per cent protein diet it rapidly subsides and has almost disappeared 5 days after grafting. In the 5 per cent protein diet animals, on the other hand, the inflammatory reaction persists for much longer and is still evident 7 days after implantation (Fig. 10).

The subsidence of the inflammatory reaction in the 20 per cent protein diet animals coincides with phase (*b*) the formation of a connective tissue capsule. This capsule begins to be evident 4 days after implantation, when cell elements of the fine connective tissue network undergo fibrosis, and the orderly arrangement of the fibrocytes makes the capsule a well defined and easily observable structure (Fig. 11).

The 5 per cent protein diet rats, however, still show persistence of the inflammatory reaction at a time when the capsule is already well developed in 20 per cent protein diet animals. The exudate around the graft remains rich in reticuloendothelial cells and fails to display any marked organization (Fig. 10). It appears that the prolonged persistence of the inflammatory reaction around the implant is related to the absence of the connective tissue capsule, the development of which is impaired or delayed in animals maintained on a diet deficient in protein.

The evidence derived from the histological investigation of muscle implants thus supports the conclusion reached from the tumour implant experiments that while maintenence of the animals on a low protein diet has no deleterious influence on the inflammatory reaction to the implant, it does interfere to a very large extent with the organization and development of the connective tissue capsule.

INFLUENCE OF DIET ON THE RESPONSE TO RADIATION OF IMPLANTED TUMOURS.

In considering the initial greater tumour growth inhibitory response to radiation shown by animals maintained on a low protein diet (Fig. 1) the question arises whether this difference in response should be attributed to a relatively greater sensitivity to radiation of the tumour cells themselves, which has been induced in some manner by the low protein diet.

It appeared possible to test this suggestion by a cytological analysis of the response to radiation of tumours in rats maintained on the different diets.

Cytological Effects.

A quantitative analysis of radiation-induced injuries in cells of the Walker carcinoma is difficult owing to the large number of chromosomes ($2n = 40$) in the relatively small tumour cell. Injuries to the chromosomes can be detected only in ana-telophase during which the lagging of "acentric" chromosome fragments (segments without the centromere) can be seen. These are caused by breakages in the chromosome filament (Fig. 12, 13, 14). Radiation damage is also indicated by the presence of dicentric chromosome bridges connecting the two daughter nuclei at telophase (Fig. 15, 16, 17). These "bridges" result when

EXPLANATION OF PLATES.

FIG. 2.—Area adjacent to tumour graft 3 days after implantation. Tumour cells have migrated out of the graft and are scattered among the fine network of the connective tissue. × 340.

FIG. 3.—Area adjacent to tumour graft 5 days after implantation. The vascular system is being established. × 340.

FIG. 4.—Developing tumour 6 days after implantation. At the periphery of the graft the number of tumour cells increases partly by migration and partly by mitosis. × 340.

FIG. 5.—Walker tumour 7 days after implantation, showing the formation of the tumour parenchyma which now obscures the connective tissue network.

FIG. 8.—Walker tumour 15 days after implantation, 5 per cent protein diet, showing the loose oedematous connective tissue tumour bed or capsule. The tumour which occupies the left hand side of the picture has no distinct boundary. × 190.

FIG. 9.—Walker tumour, 15 days after implantation, 20 per cent protein diet. The connective tissue capsule around the tumour is well organized and tumour boundary is very distinct. × 190.

FIG. 10.—Muscle implant, 7 days old, 5 per cent protein diet. The inflammatory reaction is intense around the bundles of muscle and the capsule is represented by loose, disorganized connective tissue, similar to that in Fig. 8. × 190.

FIG. 11.—Muscle implant, 7 days old, 20 per cent protein diet showing a very well organized connective tissue capsule. The inflammatory reaction is slight. × 190.

FIG. 12.—Dividing tumour cell in late anaphase, with one lagging acentric fragment, 6 hours after 100 r, 5 per cent protein diet. × 2400.

FIG. 13.—Dividing tumour cell in late anaphase with three acentric fragments, one lying out of focus, 6 hours after 300 r, 5 per cent protein diet. × 2400.

FIG. 14.—Dividing tumour cell in late anaphase showing a broken dicentric chromosome bridge; the acentric fragment remains attached to the centric segment. Below there is another acentric fragment, slightly out of focus; 12 hours after 300 r, 5 per cent protein diet. × 2400.

FIG. 15.—Tumour cell in telophase showing one bridge and three displaced chromosome fragments slightly out of focus; 12 hours after 300 r, 5 per cent. protein diet. × 2400.

FIG. 16.—Tumour cell in anaphase showing several double bridges, which indicates that the cell is undergoing a second mitosis after irradiation. No fragments could be seen; 48 hours after 100 r, 20 per cent protein diet. × 2400.

FIG. 17.—Tumour cell in anaphase showing interlocking of chromosome bridges. Several other bridges are also present, but lie out of focus; 48 hours after 300 r, 20 per cent protein diet. × 2400.

FIG. 18.—Walker tumour, 9 days old, 3 days after 1000 r, 20 per cent protein diet, showing extreme tissue fibrosis in the capsule. The tumour at the bottom of the picture is represented by a necrotic mass. × 130.

FIG. 19.—Walker tumour, 12 days old, 6 days after 1000 r, 20 per cent protein diet. The capsule is represented by a thick fibrous connective tissue which surrounds the necrotic tumour mass. × 130.

FIG. 20.—Walker tumour 9 days old, 3 days after 1000 r, 5 per cent protein diet. The capsule has undergone only slight fibrosis and contains many scattered tumour cells. At the bottom of the picture, the tumour parenchyma has broken down into a necrotic mass. × 130.

FIG. 21.—Walker tumour, 12 days old, 6 days after 1000 r, 5 per cent protein diet. The tumour bed is oedematous and shows little fibrosis. The tumour parenchyma is well demarcated and contains active tumour cells. × 130.

FIG. 23.—Walker tumour, (H_1), 20 per cent protein diet after 3000 r. The tumour boundary is sharply defined, and the connective tissue of the capsule is undergoing fibrosis. × 72.

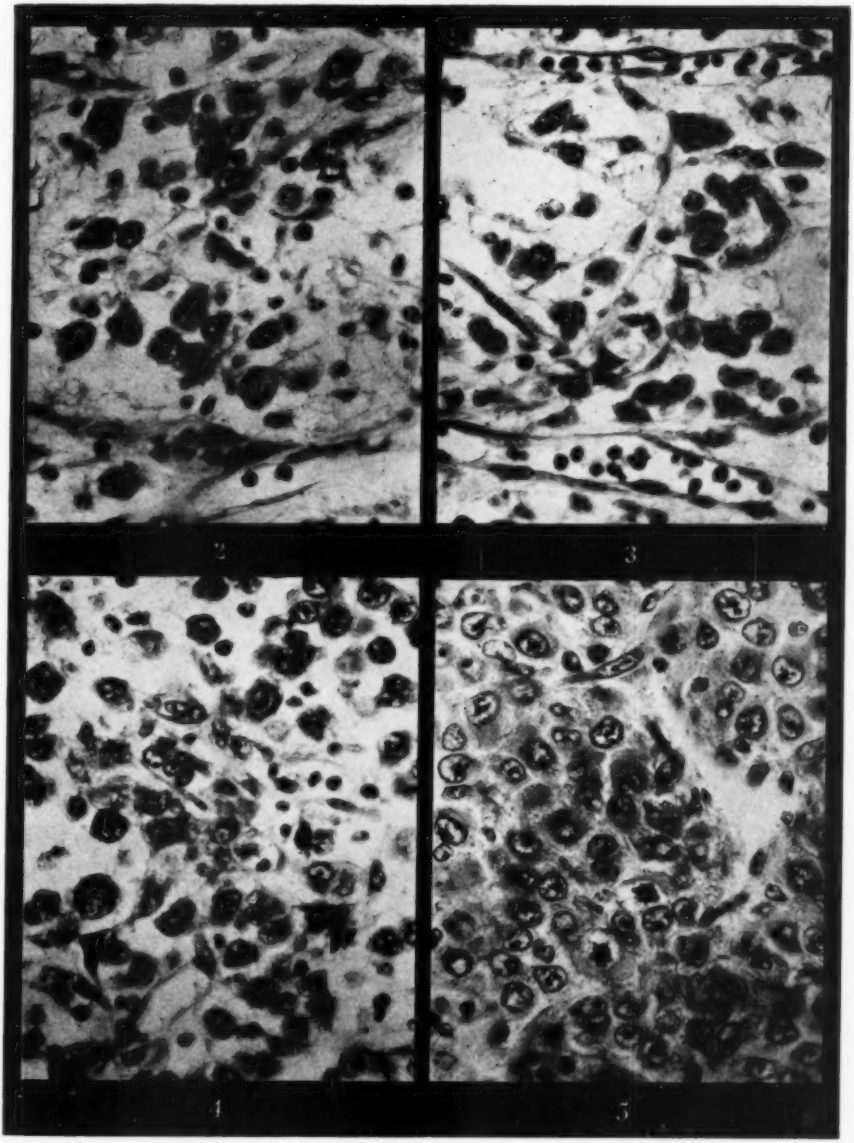
FIG. 24.—Walker tumour (L_1), 5 per cent protein diet after 3000 r. The boundary of the tumour parenchyma is irregular and the connective tissue of the capsule shows only slight fibrosis. × 72.

FIG. 25.—Walker tumour (H_2), 20 per cent protein diet after 4000 r, showing the necrotic tumour mass on the right and the well differentiated fibrous capsule. × 72. (Compare Fig. 26).

FIG. 26.—Walker tumour (L_2), 5 per cent protein diet after 4000 r. There is a certain degree of pycnotic degeneration in the tumour and fibrosis in the tumour bed. × 72.

FIG. 27.—The same tumour (L_2), showing the active tumour cell island which lies beyond the periphery of the tumour which occupies the bottom right hand corner. × 190.

FIG. 28.—The same tumour (L_2) at a higher magnification to show that tumour cells which were scattered in the loose connective tissue capsule form foci of renewed activity after irradiation. × 300.



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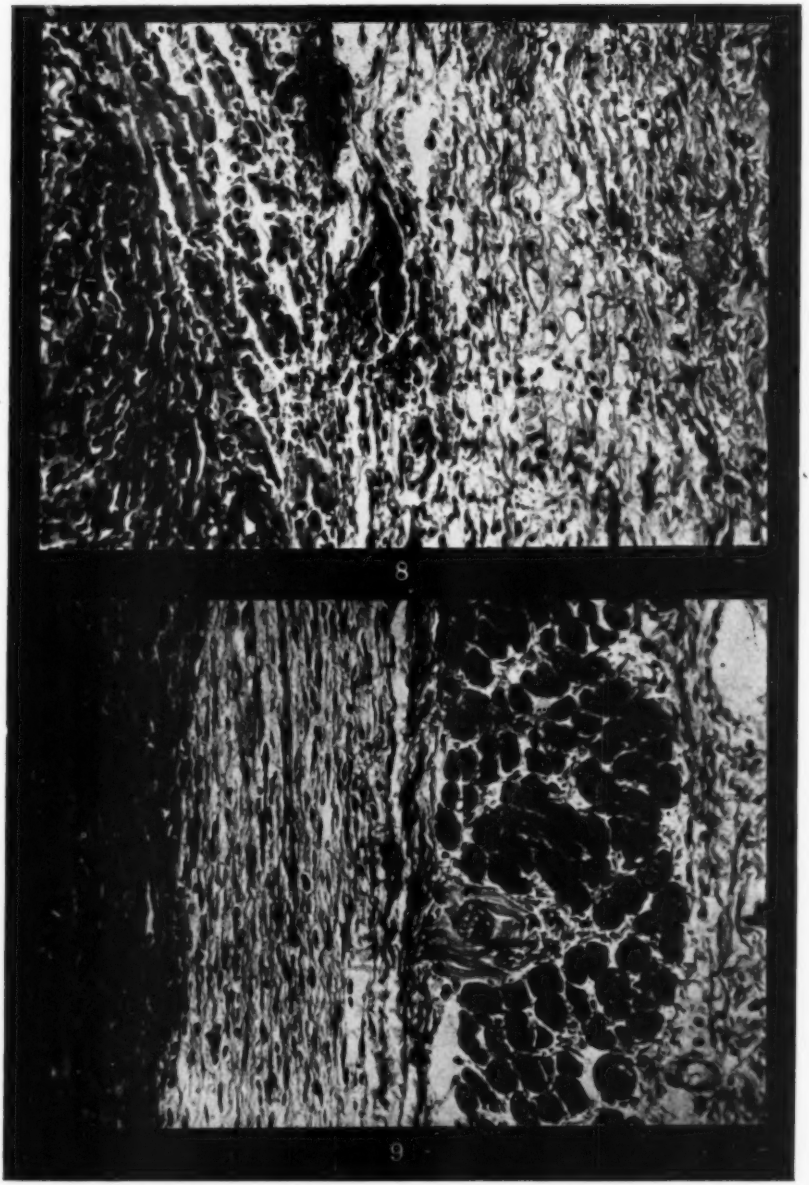

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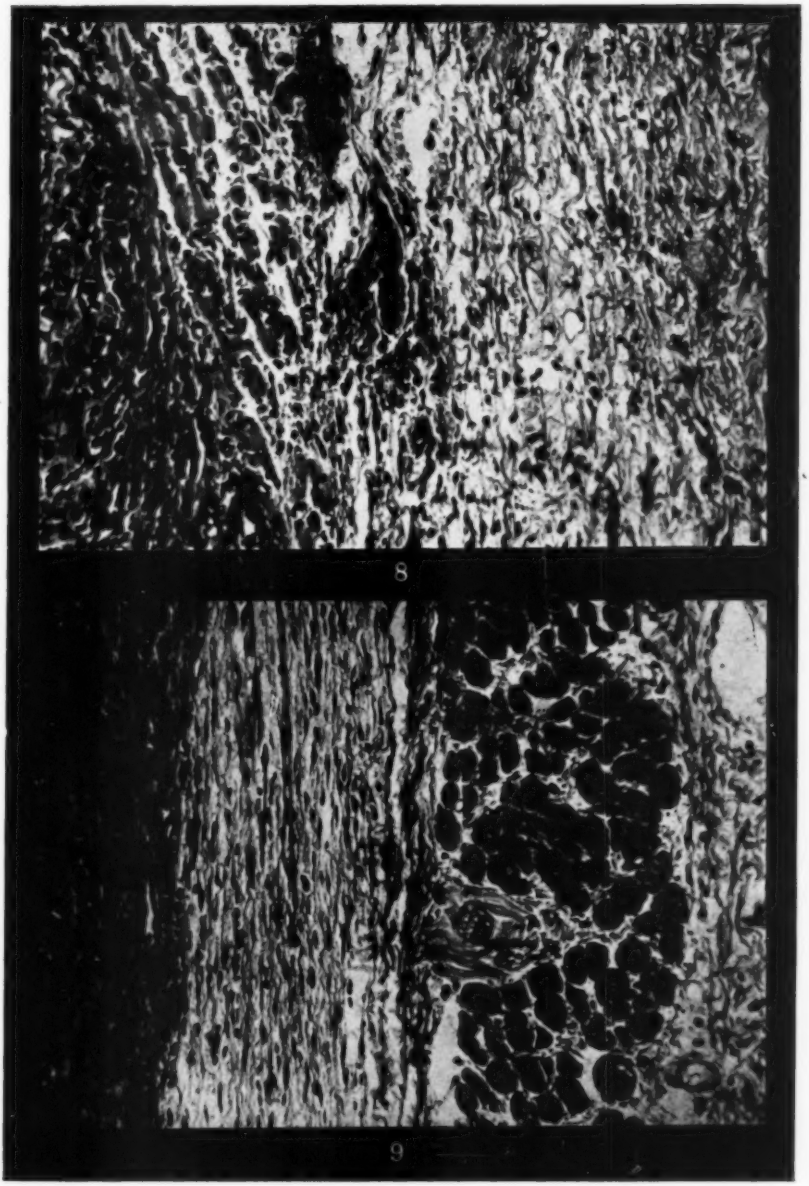

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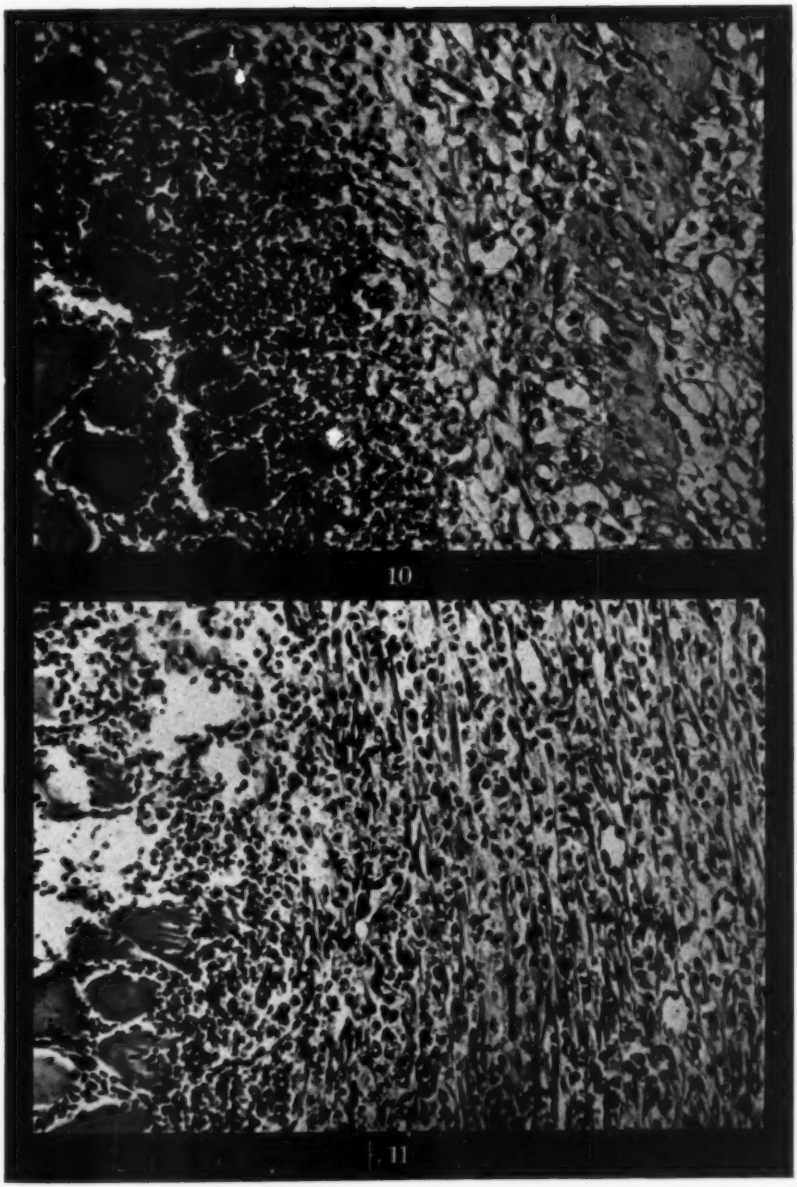

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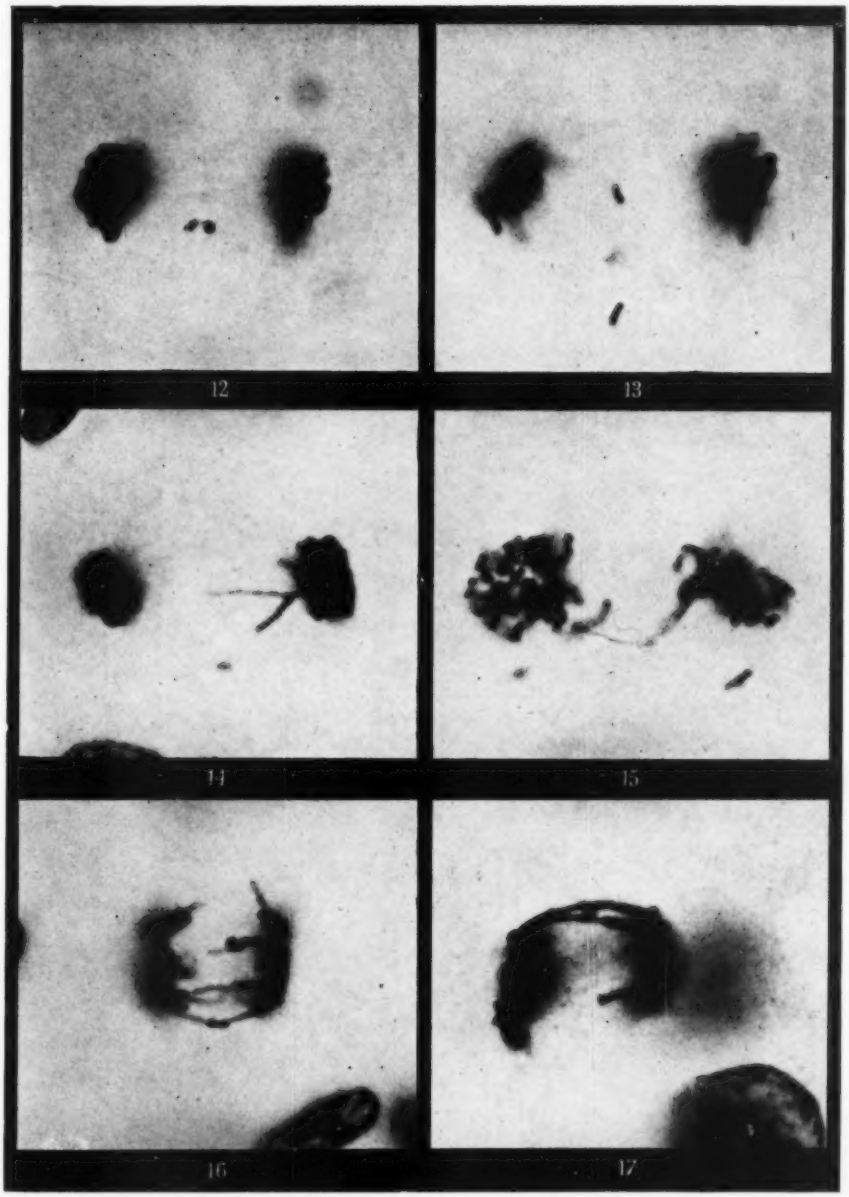

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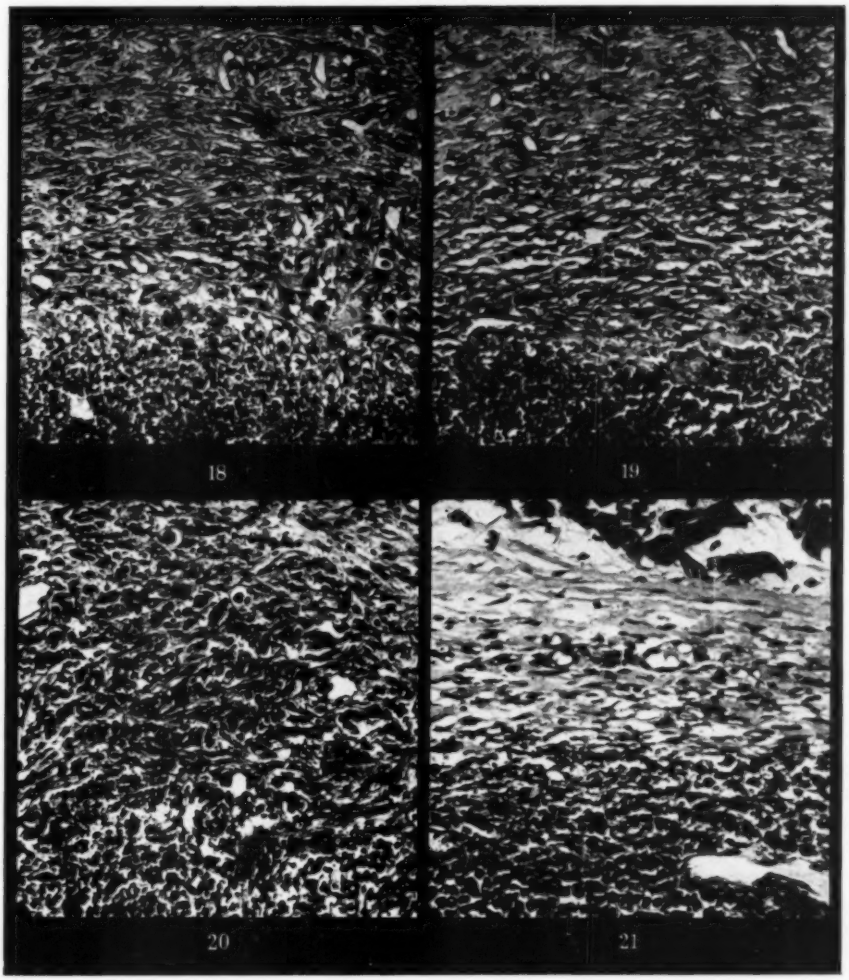

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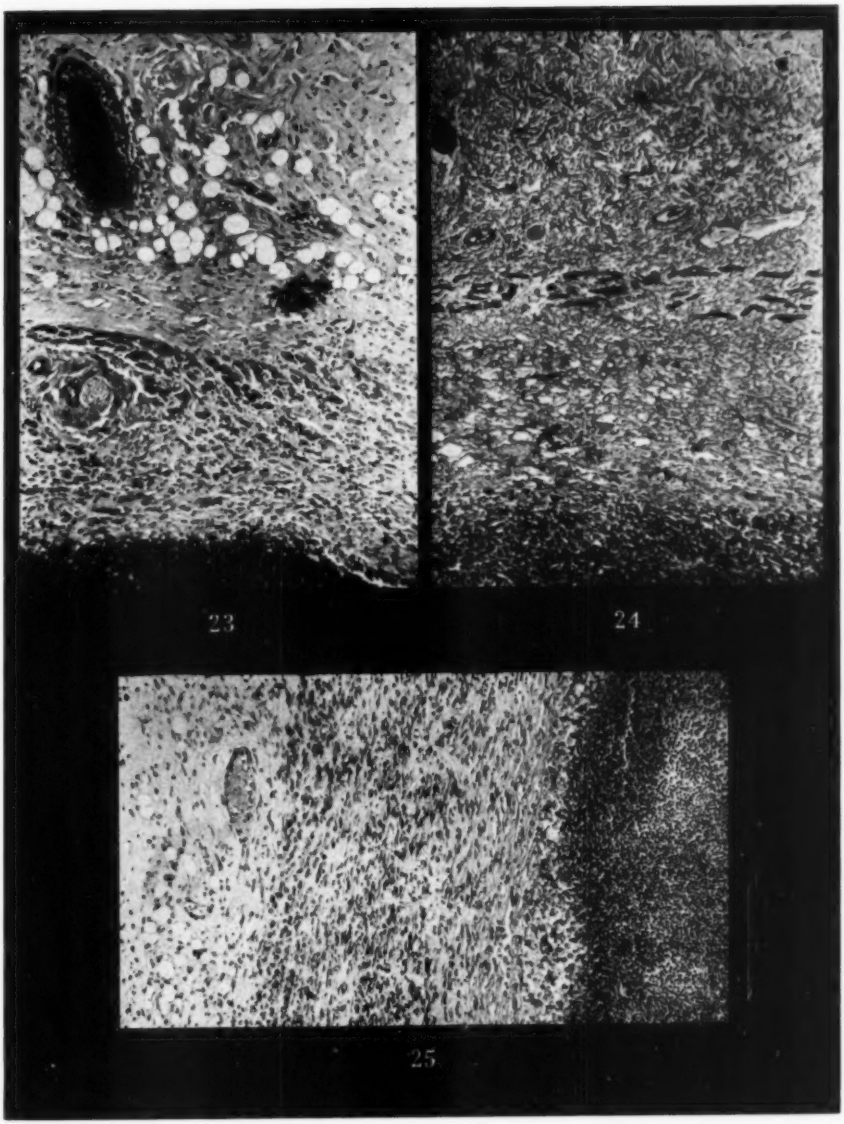

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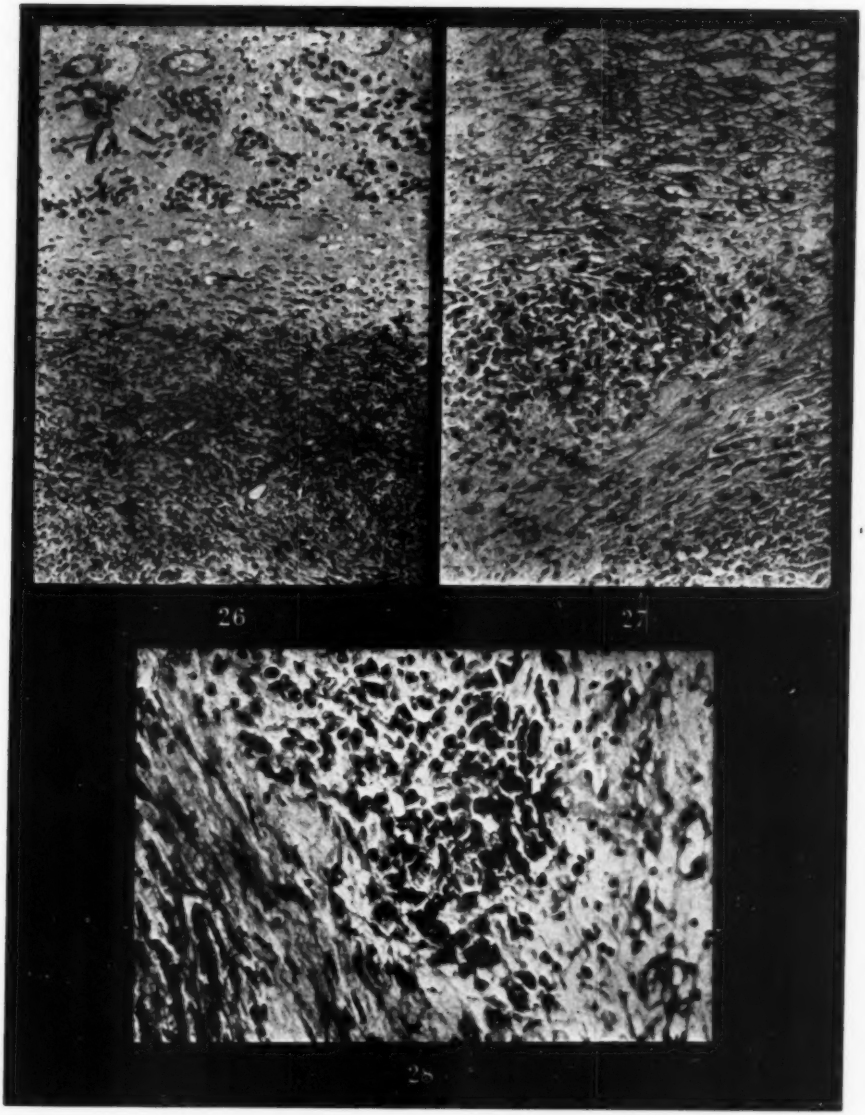

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breakages in the chromosomes are followed by a rejoining of the broken ends in a new configuration. The presence of small, supernumerary nuclei called " micronuclei " in the resting cells is a third indication of injury. These micronuclei represent acentric chromosome fragments, which were left behind in the cytoplasm at telophase, and not included in the daughter nuclei.

A comparison has been made of the frequency of tumour cells with various cytological abnormalities, such as chromosome fragments, bridges and micronuclei at different times after irradiation of a six-day old Walker tumour in rats kept on high and low protein diets. The effects of two different doses of radiation 100 r and 300 r were studied. The data obtained (Table I) do not represent the full extent of the radiation injury resulting from any particular treatment, since an unknown proportion of injuries undergo restitution before the cytological examination is made and are thus lost for analysis. Moreover, the acentric chromosome fragments can only be seen when they lie freely in the cytoplasm, and consequently those fragments which are mixed up with the normal chromosomes and moved to the pole escape detection. Such an event is indicated by the absence of fragments in cells with chromosome bridges. Thus the number of fragments seen and recorded is often less than the number of fragments originally induced. A similar difficulty arises when we attempt to estimate the true extent of the radiation injury to cells by the number of micronuclei, since some of the chromosome fragments may be included in the daughter nuclei and some of the micronuclei can contain more than one fragment. Therefore the number of micronuclei within the cell cannot be considered as a reliable criterion of the primary radiation injury. On the assumption, however, that the chance for such events is the same in all the samples, it is justifiable to use the quantitative differences shown by the data as a basis for comparing differences in cell behaviour which may be induced by maintaining the animals on different diets.

The data of Table I shows that the greatest amount of injury was found in tumour samples taken 12 hours after irradiation, suggesting that these samples contained cells which were, at the time of irradiation in the most sensitive period of the mitotic cycle. It is inferred on experimental evidence, derived from x-rayed pollen-grains and root-tips cells (Darlington and LaCour, 1945) that the most sensitive period coincides with the duplication of the chromosome filament, which process marks the beginning of prophase. Since the injury in the cells is at a maximum at 12 hours after both 100 and 300 r, we may conclude that the duration of mitotic suppression does not differ over this range of dosage.

The number of cells with chromosome injuries and with micronuclei was found to be already quite high in the 6 hours' sample, suggesting that the time interval between treatment and the first appearance of chromosome fragments may be shorter than 6 hours. Therefore some rats were killed 4 hours after treatment, but although both chromosome fragments and bridges were observed in this early sample, their frequency could not be estimated because the so-called " physiological " effects of radiation grossly interfered with the analysis (Marquardt, 1938; Koller, 1943). These physiological effects are characteristic features of cells which were in mitosis during the radiation. Such cells show stickiness and clumping of chromosomes, which makes the identification of the true chromosome bridges and fragments very difficult. For this reason the 4 hours' sample could not be used for quantitative analysis.

The number of resting cells with micronuclei increases up to 24 hours after treatment, but at 72 hours such cells have almost disappeared from the tumour. These cells, whose nucleus is deficient in chromosome material, are now seen as degenerating cells which will eventually die. Tumour cells which escaped radiation injury also now begin to divide and cause an increase in the relative number of normal cells.

Comparison of the results obtained at different times after irradiation with both 100 r and 300 r (Table I), in all samples except those taken after 12 hours, shows no significant difference in the number of cells injured or the number of chromosome fragments per cell, between animals maintained on high or low protein diets. The samples taken 12 hours after radiation, however, suggested the possibility of slightly different sensitivity (Table I, Column VIII). A repeat experiment was therefore carried out, but the results did not confirm this suggestion (Table II).

The inconsistent behaviour observed in the 12-hour samples may be related to the fact that all the mitotic cells in this sample represent those cells which were in their most sensitive state when irradiated. Cell behaviour in this stage is more readily affected by environmental factors than at other stages (Koller, 1946). Rigid control of environmental factors was not feasible since the experiments were of necessity carried out at different times and the rats used differed in age, weight, sex and genetic constitution. It was, therefore, felt that the results obtained with the 12-hour samples could not be relied upon and should be disregarded in view of the consistent results obtained with all the other samples. We may conclude, therefore, that no definite difference in the sensitivity of tumour cells to radiation has been shown to exist between rats maintained on high or low protein diets.

Since particular chromosome configurations enable us to distinguish between the first and second mitosis after X-radiation, the duration of the inter-mitotic (or " resting ") period of the active tumour cells can be estimated. It is thus possible to compare the length of the inter-mitotic period on the two diets in order to find whether the duration of the mitotic cycle is altered by the diet or not. It was found that 48 hours after 100 or 300 r some cells in mitosis showed chromosome configurations which clearly indicated that they were now undergoing a second mitosis (Fig. 16, 17). It can be assumed that cells which show the greatest amount of injury are those which at the time of irradiation were at the beginning of prophase of mitosis. In the x-rayed Walker carcinoma the greatest amount of injury is found in the 12-hour sample and consequently the duration of mitosis from prophase to the end of telophase cannot be longer than 12 hours. The cycle of the second division (from prophase to telophase) would occupy another 12 hours, thus leaving 20 hours for the duration of the inter-mitotic period, if an allowance of 4 hours is made for the time lost by the radiation-induced mitotic suppression. Thus we may conclude that the active cells in the Walker carcinoma have a mitotic cycle of the order of 32 hours (20 hours spent in resting and 12 hours in mitosis) ; this agrees with data obtained in similar experiments in which irradiation is replaced by radiomimetic chemicals, such as the nitrogen mustards (Koller, unpublished communication).

From the observations of the frequency of cells in second mitosis 48 and 72 hours after irradiation with 100 or 300 r there was no indication that diet affects the duration of the mitotic cycle.

We must conclude, therefore, that the data obtained in the cytological analysis do not demonstrate an influence of diet on the sensitivity of tumour cells to radiation.

Histological Effects.

Effect of a single dose of 1000 *r.*

In rats maintained on the high (20 per cent) protein diet it was found that 3 days after treatment with a dose of 1000 r applied direct to the tumour there was a great reduction in the number of dividing tumour cells, and at the same time a distinct process of fibrosis was in progress in the surrounding connective tissue (Fig. 18). At 6 days after the radiation treatment tissue fibrosis was much more developed, and the connective tissue capsule could be clearly distinguished from the actual tumour, since no tumour cells were present in this capsule zone. The tumour itself is enclosed within this fibrous capsule and shows widespread necrosis (Fig. 19). It does, however, contain islands of active tumour cells, which, since the growth-inhibiting effect of a single dose of 1000 r is usually only temporary, no doubt represent centres from which the renewed growth of the tumour develops.

In the animals receiving the 5 per cent protein diet, the loosely organized connective tissue capsule, 3 days after 1000 r, was not undergoing fibrosis to the same extent as the capsule in the 20 per cent protein diet animals. Very often it shows a slight oedema and contains numerous tumour cells (Fig. 20). Six days after irradiation the tumour boundary which was previously very ill-defined, becomes more distinct, presumably owing to the fact that some of those tumour cells which were scattered in the connective tissue network and have survived the radiation treatment, have now undergone proliferation and brought about the formation of a new continuous tumour parenchyma. At the periphery of the tumour numerous dividing cells are now seen (Fig. 21).

Effect of fractionated dosage (total dose 4000 *r).*

While a single dose of 1000 r may cause considerable inhibition of growth of the Walker rat carcinoma, and the inhibition may last for several days, complete regression of the tumour rarely occurs. The experiments with the single dose have shown, however, that at least in the 20 per cent protein diet animals, whilst the extent and rate of histological changes induced in the capsule or tumour bed is very favourable, it is insufficient to enable the animal to effect a cure. It is obvious, therefore, that the amount of radiation injury in the tumour cells must be increased without at the same time adversely affecting the favourable tissue response. It was therefore decided to increase the dose to 4000 r, which is of the order employed in the radiotherapy of human cancer, and a suitable method of fractionation for applying this dose was devised based on observations of the response of tumour capsule and tumour bed obtained in the single dose experiments. The method employed can be illustrated as follows : 1000 r - - - 500 r, 500 r - - - 500 r, 500 r - - - 500 r - - - 250 r - - - 250 r, in which - - - represents two consecutive days on which no treatment was given. By such treatment complete regressions have been obtained in nearly 90 per cent of the animals maintained on the 20 per cent, but in only about 15 per cent of those kept on the 5 per cent protein diet (Elson and Lamerton, 1949).

For the histological investigations 15 rats were treated with this fractionated dose, 7 of them being maintained on the 20 per cent and 8 on the 5 per cent protein diet. The growth of the irradiated tumours is represented in Fig. 22. Two rats of each diet group H_1, H_2 (High protein) and L_1, L_2 (low protein) were selected for histological examination ; of the remaining rats 4 out of the 5 main-

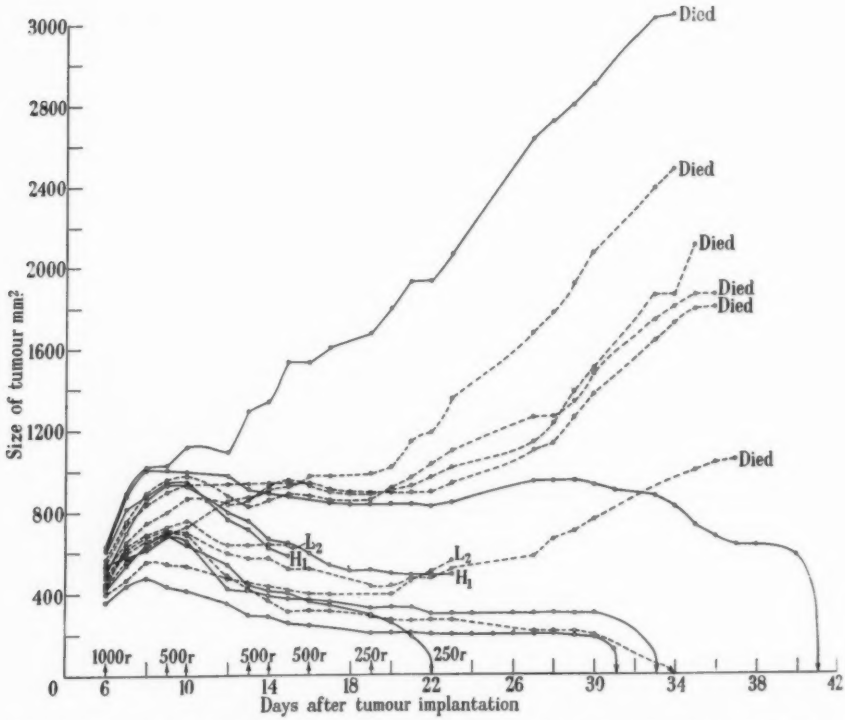

FIG. 22.—Growth of Walker tumour treated with X-radiation ; extended treatment with initial high dose (1000 r) ; total dose 4000 r. H_1 and H_2 indicate animals maintained on high protein diet and L_1 and L_2 animals maintained on low protein diet at the time they were killed for histological examination. ——— Rats fed on high (20 per cent) protein diet ; - - - - - rats fed on low (5 per cent) protein diet.

tained on the 20 per cent protein diet were cured, whilst only one cure was effected in the group of 6 animals receiving the low protein diet. From similar results obtained in previous experiments (Elson and Lamerton, 1949) and from a comparison of the growth curves of the tumours selected for histological investigation with those of the remainder of the groups shown in Fig. 22 it is reasonable to suppose that animals H_1 and H_2 on the high protein diet would have been cured, whereas L_1 and L_2 maintained on the low protein diet would not.

The tumours of the animals maintained on both diets were about the same size when the animals were killed for histological examination. Those of the high protein diet animals (H_1 and H_2), however, had grown to a considerable maximum size and were then regressing, whilst those of the low protein diet animals (L_1 and L_2) had shown earlier retardation of growth, but were beginning to assume a more rapid growth rate. The animals H_1 and L_1 had received a total dose of 3000 r (in 5 fractions), whereas H_2 and L_2 had received the full total dose of 4000 r (in 8 fractions).

In the rat H_1 the connective tissue capsule round the tumour was found to have undergone fibrosis, forming a very distinct boundary to the tumour. The solid continuous tumour parenchyma showed widespread cell degeneration, indicating that serious injury had been inflicted on a great number of tumour cells (Fig. 23). In the low protein diet animal L_1, on the other hand, numerous tumour cells were found scattered throughout the capsule zone in which fibrosis was much less marked than in H_1, thus making the tumour boundary very ill-defined (Fig. 24).

These differences between animals maintained on high and low protein diets became more marked after the full dose of 4000 r. In animal H_2 extremely intense fibrosis of the capsule was found, the damaged tumour tissue was disintegrating, and reabsorption of the necrotic tumour mass was taking place; tumour destruction and elimination is in fact in progress and should eventually lead to cure of the animal (Fig. 25).

In the low protein diet animal L_2 the tumour cells constituting the parenchyma had undergone " over differentiation " and pycnotic degeneration as a result of radiation injury to the chromosomes (Fig. 26). In the loosely fibrosed capsule zone, however, those tumour cells which were either undamaged or had managed to recover from the radiation injury were beginning to form islands of new tumour growth (Fig. 27, 28).

DISCUSSION.

In assessing the response of tumours to radiation two effects must be considered: (1) The initial inhibition of tumour growth and (2) the elimination of the inhibited tumour and cure of the animal. In work with the Walker rat carcinoma 256 it has previously been shown that the diet of the animal has a definite influence on each of these processes (Elson and Lamerton, 1949). The object of the present investigation was to determine the biological basis of these diet effects.

Dealing first with process (1), it has been shown that maintenance of the animal on a low protein diet favours the immediate growth inhibitory response of the implanted Walker rat carcinoma 256 to radiation. It might be thought that this diet effect is related to an increased sensitivity of dividing tumour cells to radiation induced in some manner by the poor protein diet. The cytological investigation reported here has, however, yielded no evidence of any such increased sensitivity. Furthermore, we do not feel that such differences as have been revealed in the course of the histological investigation of the tumour bed reported here offer any very satisfactory explanation of the greater immediate tumour response in the low protein diet animals, and we are inclined to believe that some more fundamental biochemical relation is involved.

Investigations with carcinogenic chemicals have suggested that these substances may inhibit cellular growth by interfering with the normal processes of protein synthesis (Elson and Warren, 1947 ; Elson, 1949). If this is the case it might be expected that animals in which the full capacity for protein synthesis is already restricted by a deficient protein diet would probably show an immediate growth inhibitory response of both animal and tumour to treatment. Animals which in their diet were supplied with ample protein to furnish more than sufficient amino acid " building blocks " for the full efficiency of protein synthesis would not be expected to show such an immediate reaction. It is not unlikely that X-radiation may act in a similar way and in order to throw more light on this problem the protein metabolism of irradiated animals maintained under controlled nutritional conditions is now being investigated.

Considering now process (2) the histological investigations reported here suggest a feasible explanation of the dietary effects. In the radiation treatment of the Walker rat carcinoma a high protein diet has been shown to be of great assistance to the animal in the process of elimination of the inhibited tumour and the replacement of the tumour area by healthy tissue. The histological analysis has revealed that in the case of the implanted Walker rat carcinoma 256 the main effect of the high protein diet is to ensure the development of a well organized, extensive capsule of connective tissue around the growing tumour. In an untreated tumour this capsule, particularly in the earlier stages, may assist the growth of the tumour by forming a " tumour bed " well equipped to supply its nutritional requirements. Under the action of radiation, however, the natural tendency of the connective tissue capsule towards fibrosis becomes much enhanced. Thus the tumour is now enclosed within a firm fibrous capsule and, owing to the cellular damage caused by the radiation, breaks down into a necrotic mass and will eventually be eliminated and replaced by healthy tissue.

Animals maintained on a low protein diet usually fail to form such a well developed tissue capsule round the tumour implant. The tumour is thus more diffuse and although it may at first show a marked response to radiation treatment the ill-defined tissue capsule zone does not undergo fibrosis in the same way as does the firm capsule of the high protein diet animals. The tumour may stop increasing in size, but does not usually undergo marked regression. Although its main mass usually becomes necrotic, new tumour growth often develops in the incomplete capsule zone, from cells which have escaped the lethal action of the radiation.

Hence it appears that some factor or factors required for the efficient development of a connective tissue capsule are not provided in adequate amounts if the animals are maintained on a diet deficient in protein. The effect of addition to the low protein diet of substances such as amino acids, particularly cystine and methionine, amino sugars, etc., which may conceivably be involved in the process of connective tissue formation is being investigated. A knowledge of how connective tissue reactions are influenced by dietary factors clearly has an importance well beyond the field of investigation reported here.

Although our investigation has so far only been carried out on a particular animal tumour, the data obtained have a bearing on the radiotherapy of human cancer, and in particular emphasize further the importance of the tumour bed reaction, already revealed by previous work. Koller and Smithers (1946),

taking into consideration the connective tissue reaction, devised treatment methods for epitheliomata, in which the total dose is of the order of 3000 r, far below the so-called "standard tumour lethal dose." Also it has been shown by Jolles and Koller (1950) that the tumour bed reaction can be influenced by a method of fractionation of the dose in space as well as time to give favourable therapeutic results.

The present animal experiments have shown that the character and organization of the tumour bed can be influenced by nutritional factors, and suggest that suitable regulation of nutritional conditions, such as, for instance, supplying a high protein diet, or supplementing the diet by protein hydrolysates, etc., and by increasing protein anabolism by hormone administration, etc., may lead to improvements in clinical response.

SUMMARY.

The effect of the protein content of the diet on the establishment and growth of implants of the Walker rat carcinoma 256, and on the response of this tumour to X-radiation, has been investigated.

A subcutaneous graft of tumour tissue causes an inflammatory reaction to develop around the implant. This is followed by the formation of a capsule of connective tissue into which tumour cells from the implant migrate and undergo mitosis, thus establishing the growing tumour.

The nature and extent of the inflammatory reaction has been found to be similar in animals maintained on both high (20 per cent) and low (5 per cent) protein diets, but in the low protein diet animals it persists for a longer period after implantation owing to slower and less complete development of the connective tissue capsule.

In assessing the response of the tumour to radiation two effects have been considered, (*a*) the initial inhibition of tumour growth, which is favoured by a low protein diet, and (*b*) the elimination of the inhibited tumour which is favoured by a high protein diet.

Cytological investigation has revealed no evidence of increased sensitivity to radiation of tumour cells in animals maintained on a low protein diet.

Histological investigation has suggested that the favourable effect of a high protein diet on the elimination of the tumour is related to the development of the well organized capsule of connective tissue around the growing tumour. Radiation treatment, besides inhibiting division of the tumour cells, also increases the fibrosis in this capsule, which aids in the eventual elimination of the damaged tumour.

These investigations draw attention to the important role of tumour environment in the radiation treatment of cancer.

We wish to express our thanks for grants supporting this investigation from the British Empire Cancer Campaign, the Anna Fuller Fund, the Jane Coffin Childs Memorial Fund for Medical Research and the U.S. Public Health Service; also for facilities provided by Imperial Chemical Industries Limited.

Experiment II in Table II was done by Mr. S. H. Revell (May, 1949), to whom we are greatly indebted.

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A SEX DIFFERENTIAL FOR CHEMICALLY INDUCED FIBROSARCOMA ASSOCIATED WITH LITTER SERIATION.

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Received for publication May 23, 1950.

DATA have been published which indicate that the average latent period for all methylcholanthrene-induced fibrosarcomas (Strong, 1948) and the survival time (Strong, 1950) of mice developing such tumours are both influenced by litter seriation. The latent period (time between the subcutaneous injection of the carcinogen and the initiation or the initial growth of the ensuing fibrosarcoma) was variously affected in the different strains by the litter to which the mouse belonged. In mice of some strains (prunt and F_1 of $C_{67} \times$ Brs) the latent period (Strong, 1948) for the appearance of fibrosarcomas decreased in the successive litters of the same pair of mice, while in other strains susceptibility to fibrosarcomas was relatively constant. Unpublished data have also disclosed that in one subline of mice the average latent period of fibrosarcomas increases in mice of the successive litters. Thus a variable mechanism is indicated in the different sublines which influences susceptibility to fibrosarcomas, and this mechanism is associated with litter seriation. The survival time (Strong, 1950) of mice growing a chemically-induced fibrosarcoma (time between the initial growth of the malignancy and the death of the individual) is also affected by litter seriation. When a mouse developed a fibrosarcoma in less than 100 days following the subcutaneous injection of methylcholanthrene it survived an average of 52 days with the progressive growth of the tumour if it belonged to a first litter. In the succeeding litters mice of the same age and latent period survived longer and longer with growing fibrosarcomas until, if they belonged to an eighth litter, they lived on an average of 130 days.

In this paper data on the average percentage incidence of methylcholanthrene-induced fibrosarcomas are given for mice which develop their malignancy in less than 100 days. Data on the sexes of the mice are separated.

The present observations have been made on two sublines of the pBr strain of mice. The origin of these sublines will only be discussed briefly here. The pBr subline was separated from the original NHO descent in the F_{14} generation of sib inbreeding. The mice are marked by the genes for pink eye, brown and non agouti (genetic constitution ppbbaa). These genes were derived from mice of the ancestral stocks JK and N which were used in the origin of the NHO strain. The first three generations (CBAN $\times$ JK) were used for breeding purposes only. From the F_4 generation onward for several generations both parents were injected with 1 mg. of methylcholanthrene at 60 days of age. In the F_{17} generation of continued brother-to-sister matings four mice (born 1.xi.45) were set aside

from the experimental animals receiving methylcholanthrene, and have been continued as an untreated descent ever since. These mice are now in the F_{30} generation. This untreated descent has been given the symbol prunt. At the time of the separation of prunt from pBr descent, the average latent period for the appearance of methylcholanthrene-induced fibrosarcomas of the direct maternal ancestry was 369·5 days. In the F_{20} generation of the experimental pBr descent receiving methylcholanthrene, a single pair of mice was set aside and continued as a second untreated descent. This second group received the symbol 2prunt. At the time of separation the average latent period for methylcholanthrene-induced fibrosarcomas of the direct maternal ancestry was 393·0 days. The original mice of the 2prunt descent were born 14.vii.47. At present this 2prunt descent is in the F_{28} generation of inbreeding. These two untreated descents, prunt and 2prunt, should possess genetic similarity, and any biological differences that they have may be due either to divergent segregation from a residuum of genetic heterozygosity (probably very small after 17 generations of brother-to-sister matings) or to new biological variability which has appeared in one or both untreated descents following their separation from a common ancestry.

At 60 days of age the offspring of the untreated prunt and 2prunt sublines were injected subcutaneously with 1·0 mg. of methylcholanthrene dissolved in 0·1 c.c. of sesame oil. All mice were kept in an air-conditioned laboratory with temperature controlled between 70 and 73° F. and the humidity between 50 and 60 per cent. The mice were fed a standard diet of Nurishmix pellets (Pratt Food Company) and water *ad libitum*. A supplement of mixed grains (oats, wheat and sunflower seeds) and calf meal pellets were given to the mice once a week. Enriched Bond bread soaked in milk and Squibb's cod-liver oil was also given once a week. The mice were examined periodically for tumours, and the latent period taken to be the time at which a *firm* nodule at the site of the injection of the carcinogen began to increase progressively in size. Mice which developed tumours at sites other than the one at which the carcinogen had been injected were tabulated separately. A determination of histological types of tumours was obtained by saving tissues from the tumour at the time of the autopsy of the mouse.

Table I gives the percentage incidence of tumours in mice which developed in less than 100 days (Column *e*), together with the data obtained on all tumours at the site of injection of the carcinogen irrespective of latent period (Column *d*). Data on the total number of mice injected with methylcholanthrene (Column *a*), the number of mice which died without developing any tumour (Column *b*), and the total number of mice showing tumours irrespective of latent period and site are also given (Column *c*). Since the two separate descents disclose similar genetic origin and show similar trends by which the females are consistently more susceptible to fibrosarcomas than the males, the data for both series may be added together. However, the present analysis of trends of susceptibility in successive litters discloses differences, and therefore the two strains, the prunt and the 2prunt, should be kept distinct. The data contained in Table I for the prunt descent, Column *e*/Column *a*, are given graphically in Fig. 1.

Up to the present time 477 mice of the prunt descent have been injected with methylcholanthrene. Of these, 250 were females and 227 were males; 337 have developed fibrosarcomas at the site of the injection and have died. Of this total,

TABLE I.—*Incidence of Tumours.*

Stock.	Litter.	*a* Total injected.		*b* Died, no tumour.		*c* Mice + tumours.		*d* Local tumours.		*e* Local tumours <100 days.		%. e/a.		%. e/d.	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
prunt	1	26	35	1	3	25	32	18	18	4	5	15·4	14·3	22·2	27·8
	2	38	40	3	2	35	38	32	22	8	3	21·1	7·5	25·0	13·6
	3	39	44	4	6	35	38	33	33	8	3	20·5	6·8	24·2	9·1
	4	58	43	4	5	54	38	44	29	17	3	29·3	7·0	38·6	10·3
	5	39	37	1	6	38	31	28	25	12	6	30·8	16·2	42·9	24·0
	6	31	14	0	1	31	13	21	11	11	1	35·5	7·1	52·4	9·1
	7	14	12	0	0	14	12	12	8	7	0	50·0	0·0	58·3	0·0
	8	5	2	0	0	5	2	2	1	1	0	20·0	0·0	50·0	0·0
2prunt.	1	33	26	0	2	33	24	24	16	6	3	18·2	11·5	25·0	18·8
	2	34	25	1	3	33	22	24	15	13	2	38·2	8·0	54·2	13·3
	3	62	48	2	4	60	44	37	29	8	4	12·9	8·3	21·6	13·8
	4	60	42	0	2	60	40	36	27	15	3	25·0	7·1	41·7	11·1
	5	51	51	0	5	51	46	32	30	12	5	23·5	9·8	37·5	16·7
	6	14	14	2	1	12	13	5	6	2	2	14·3	14·3	40·0	33·3
	7	18	15	2	1	16	14	10	5	5	0	27·8	0·0	50·0	0·0
	8	16	10	1	3	15	7	10	6	4	0	25·0	0·0	40·0	0·0

190 were females and 147 were males. Eighty-nine mice have developed fibrosarcomas at the site of injection with latent periods <100 days and have died. Of these, 68 were females and 21 were males. Thus 27·2 per cent of the females developed fibrosarcomas within less than 100 days of latent period, whereas only 9·2 per cent of the males developed similar tumours during the same period, a difference of 18·0 per cent in tumour susceptibility.

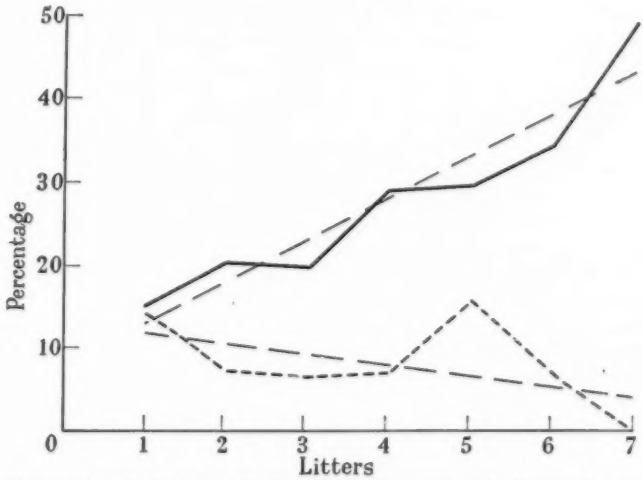

FIG. 1.—Litter seriation is plotted on the base line and percentage incidence of fibrosarcomas with latent periods less than 100 days on the vertical line. Data for females on the solid line and data for males on the short dash line. Straight lines determined by the system of least squares are drawn through the points for successive litters.

In the statistical analysis of the prunt results straight line trends were developed from the observed data by the method of least squares (Fig. 1). Then, the equations for these trends were computed. The trend for the percentage incidence of tumours developing in less than 100 days among females of successive litters was found to be $Y = 8{\cdot}5 + 5{\cdot}1x$, with a standard deviation of the slope of $\pm 0{\cdot}741$. Thus, with each successive litter the tumour incidence among females increased by $5{\cdot}1 \pm 0{\cdot}741$ per cent. Comparison of this value with a no-trend or 0-slope value by t test reveals that the observed trend is statistically significant (since $P = < 0{\cdot}01$).

Similarly the trend for the percentage incidence of tumours developing in less than 100 days among males of successive litters was found to be $Y = 13{\cdot}2 - 1{\cdot}2x$, with a standard deviation of the slope of $\pm 0{\cdot}968$. Thus, with each successive litter the tumour incidence among males decreased by $1{\cdot}2 \pm 0{\cdot}968$ per cent. This trend is not significant (since $P = > 0{\cdot}05$).

Therefore the conclusion is warranted that in the prunt descent there is a progressively increasing sex differential in tumour development in response to methylcholanthrene in mice of successive litters. This sex differential results from a statistically significant increasing susceptibility of the females in successive litters, while the male susceptibility under the same circumstances shows no significant change.

A similar analysis of the data for the 2prunt descent shows no significant increasing or decreasing sexual differential in the succeeding litters.

The analysis of the data thus discloses that there is an increasing sex differential in relation to chemically induced fibrosarcomas in successive litters in mice of the prunt descent. This is due to an increased susceptibility in the female. This however apparently is not the case in mice of the 2prunt descent. Both descents have had a common ancestry for 17 brother-to-sister matings. Thus the two descents should be considered as belonging to the *same* inbred strain of mice. The biological difference between mice of the two descents as indicated in this paper may therefore not be genetic. It is known that methylcholanthrene changes cancer susceptibility. Mice of the two untreated pBr descents (prunt and 2prunt) used in this investigation were freed from the injection of methylcholanthrene for at least 5 generations. It does not seem possible, however, that the effects of methylcholanthrene could be transmitted through 5 generations without having a change in genetic constitution. The difference between the mice of the two descents must, therefore, be associated with a mechanism which changes in litter seriation at least in some strains of mice. In the 2prunt descent the maximal sexual differential is found in mice of the second litters (30·2 per cent), and this sex differential fluctuates in the succeeding litters and thus shows no significant trend. However, the data of the 2prunt descent are complicated by a high value for females of the second litter (38·2 per cent) and a high value for males of the sixth litter (14·3 per cent). Further data may alter the present interpretation for the 2prunt descent.

DISCUSSION.

Rarely has a sex difference on chemically induced fibrosarcomas been observed. During the past ten years with the use of large numbers of mice injected with methylcholanthrene, only two previous cases have been observed. One of these

sex differences was encountered by injecting methylcholanthrene into males and females of 15 different inbred strains of mice. Of these 15 there were no sex differences in the data for 13 of the strains. In one strain (the CHI) males were more susceptible to chemically induced fibrosarcomas than were the females, whereas in the 15th inbred strain (the $C_{12}I$) the females were more susceptible to tumours than were the males. A reciprocal cross between mice of these two strains which showed sex differences to fibrosarcoma susceptibilities produced mice which had differential susceptibilities in the two F_1's. Thus evidence was obtained that this sex difference existed in the mice before the injection of methylcholanthrene. In the second sexual difference (found in the pBr treated strain) evidence was obtained that the mechanism which later gave a sexual differential was not detectable in mice of the original inbred strain, but developed subsequently to the injection of methylcholanthrene. This sex difference in relation to chemically induced tumours gradually increased in the succeeding generations of mice following its first appearance.

The mechanism involved in the development of this new sexual differential in relation to chemically-induced fibrosarcomas in mice of the prunt descent gives a slight sex difference in mice belonging to the early litters of a breeding female, and apparently gradually increases in the succeeding litters. There is abundant evidence that sex is determined by chromosomes or genes. If the sex pattern of an individual is the reflection of the genetic constitution of the individual, then it is not clear whether mice of the early litters or mice of the later litters are to be considered the pattern for the species. But sex physiology is also under the influence of several hormones, and the sex pattern can be influenced significantly when a hormone is administered at an early age, preferably before birth.

The present investigation with methylcholanthrene has disclosed that several aspects of malignancy (the average latent period, the survival time and the percentage susceptibility of tumours in mice of latent periods of less than 100 days) are influenced by litter seriation. It is therefore clear that a biological field is indicated which up to the present time seems to be woefully neglected. Do hormones fluctuate in the female body with advancing litter frequency, pass the placental barrier and influence the subsequent physiology of the offspring at least in relation to malignancy and perhaps to other characteristics as well? Or are we to conclude that maturation phenomena in cytoplasm (perhaps the mitochondria or other constituents) are responsible for this transmission from mother to offspring which apparently is not through the genes? Perhaps another biological reason may eventually be found for the data at hand.

SUMMARY.

Six hundred and forty-nine mice have had fibrosarcoma at the site of injection of methylcholanthrene and subsequently died. Of these, 368 have been females and 281 males. A hundred and seventy-three of the total number of mice have had fibrosarcomas with latent periods of less than 100 days. Of these, 133 were females and 40 males. These mice belonged to two separate descents derived from a common genetic origin. In one descent, the prunt, there was an increasing sexual differential in relation to chemically-induced fibrosarcomas in the succeed-

ing litters born to the same parents due to an increased susceptibility in the female. An increasing sexual differential was not encountered in mice of the 2prunt descent.

This experiment has been made possible by grants from The Anna Fuller Fund and The Jane Coffin Childs Memorial Fund for Medical Research. Acknowledgment is also made to Dr. H. Auerbach for the statistical analysis of the present data.

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THE EFFECTS OF A GRADED SERIES OF RESTRICTED DIETS ON EPIDERMAL MITOTIC ACTIVITY IN THE MOUSE.

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Received for publication August 12, 1950.

It has recently been demonstrated that the most critical factor determining the epidermal mitosis rate in the adult mouse is the carbohydrate supply to the cells, and the evidence at present available all points to the conclusion that the function of this carbohydrate is to supply the energy needed during cell division (Bullough, 1949*a*, 1950*a*, *b*). In extending this work the discovery has been made that a restricted diet has a powerful effect in depressing mitotic activity (Bullough, 1949*b*), and it has been suggested that this observation may help towards an explanation of Tannenbaum's remarkable results on the effects of restricted diets on tumour genesis (Tannenbaum, 1940*a*, 1940*b*, 1942, 1944, 1945, 1947).

In order to make possible a still closer comparison between the effects of restricted diets on epidermal mitosis and on tumour genesis the present series of experiments was planned. This involved a study of the results obtained with a graded series of diets as regards body weight, blood sugar concentration, liver glycogen content, and epidermal mitotic activity.

MATERIAL AND METHODS.

The mice.

The two experiments performed involved a total of 55 mice, which were all adult males of between 3 and 5 months of age. In the first experiment the 25 animals included 10 Strong's CBA males and 15 Kreyberg's white label males. In the second experiment all the animals were Strong's CBA males.

These mice had been reared since weaning on a mixed diet of commercial rat cake with cod-liver oil, flaked maize, and dog biscuit, but during the experiments they were fed on commercial rat cake alone. They invariably received their food between 09.00 and 10.00 hours, so that they formed the habit of being awake and active at that time and of being asleep in the early afternoon. This determined the form of their diurnal cycle of mitotic activity, and ensured that a high mitosis rate developed shortly after midday (Bullough, 1948). The experiments were carried out during the winter and early spring, and the animals were exposed to normal daylight.

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The diet experiments.

The commercial rat cake fed to the mice during the experiments had the following approximate composition:

Protein	19·2
Fat	4·9
Carbohydrate	52·7
Total ash	6·0
Crude fibre	4·8
Water	12·4

Preliminary work established that the average intake of this rat cake was 3·6 g. per mouse per day. In the first experiment the 5 control animals were each given this amount of food (100 per cent diet). The experimental animals, arranged in groups of 5, were given respectively 3·2 g. (90 per cent diet), 2·8 g. (80 per cent diet), 2·4 g. (70 per cent diet), and 2·0 g. (60 per cent diet). In all cases the animals had access to abundant drinking water.

In the second experiment the mice were also arranged in groups of 5, and the members of each group received respectively 3·8 g. per mouse per day (105 per cent diet), 3·4 g. (95 per cent diet), 3·0 g. (85 per cent diet), 2·6 g. (75 per cent diet), 2·2 g. (65 per cent diet), and 1·8 g. (55 per cent diet). Again abundant drinking water was provided.

Methods of observation.

All the animals were weighed at the beginning of the experiments, at weekly intervals, and on the day when they were killed. It was unfortunate that the animals in the first experiment were considerably lighter than those in the second experiment, but it was remarkable how little this fact affected the results obtained.

On the day when each experiment began a piece of ear was removed from each animal at 14.00 hours, the normal time of maximum epidermal mitotic activity associated with the afternoon sleep period. At weekly intervals further pieces of ear were taken at 14.00 hours.

In all cases the experiments continued for four weeks. On the day when the mice were killed each animal was injected at 10.00 hours with 0·1 mg. colchicine in 0·25 ml. water in order that those mitoses developing in connection with the afternoon sleep period should be arrested in the metaphase. The animals were killed at 15·00 hours, it having been previously determined that for a period of about 5 hours after the injection of this weight of colchicine there is no great disturbance of the mitosis rate or of the blood-sugar level (Bullough, 1949*c*).

Death was by chloroform, and immediately afterwards two samples of blood, each of 0·1 ml., were taken from each animal. The estimation of the blood-sugar concentration was by the Hagedorn and Jensen technique. The liver was removed, cut into two roughly equal parts, and the glycogen content was converted into glucose by the method of Good, Kramer and Somogyi (1933). The glucose was then estimated by the Somogyi-Schaffer-Hartmann method (Somogyi, 1930), and the results are expressed as mg. glucose per 100 g. fresh liver.

In estimating the epidermal mitotic activity the pieces of ear, fixed in Bouin's alcoholic fluid and embedded in ester wax (Steedman, 1947), were cut into sections 7μ thick. After staining with Ehrlich's haematoxylin, the numbers of mitoses

present were counted in unit section lengths of 1 cm. In the earclips taken at weekly intervals mitoses in all phases of division were included in the counts, but in those taken at death, after treatment with colchicine, only mitoses in the metaphase were counted. From each earclip ten counts were made, and the average taken. A grand mean and standard error was then calculated for each experimental group by the method suggested for small samples by Simpson and Roe (1939).

RESULTS.

Changes in body weight.

In the first experiment each group contained both CBA and Kreyberg's white label males, which were all 3 months old when dieting began. Their average weight was about 25 g., which is normal for well-fed males of these strains and this age. The changes in weight occurring during the experiment are shown in Table I.

TABLE I.—*The Average Body Weights (in grammes) in Groups each of 5 Adult Male Mice Fed on Restricted Diets of Rat Cake.*

Weeks.	Diets expressed as percentages of normal food intake.				
	60%.	70%.	80%.	90%.	100%.
0	24·9±1·23	25·5±0·97	25·2±0·68	24·7±1·50	25·0±0·97
1	21·3±1·02	21·7±0·96	24·4±0·52	25·7±1·18	26·1±0·85
2	18·4±1·16	20·0±0·76	21·8±0·43	25·2±1·30	26·7±0·75
3	18·1±1·17	19·9±0·75	21·4±0·52	25·3±0·75	25·4±0·92
4	18·0±0·92	19·7±0·78	21·2±0·65	24·6±0·70	27·9±0·97

In the second experiment only Strong's CBA males were used, and these, being 4 months old when the food restrictions began, were heavier than the mice of the first experiment. Their weight changes are shown in Table II.

TABLE II.—*The Average Body Weight (in grammes) in Groups each of 5 Adult Male Mice Fed on Restricted Diets of Rat Cake.*

Weeks.	Diets expressed as percentages of normal food intake.					
	55%.	65%.	75%.	85%.	95%.	105%.
0	34·8±0·67	34·8±1·10	34·0±0·97	29·7±0·76	28·9±1·50	30·6±1·16
1	29·4±0·47	30·0±1·00	29·5±1·04	26·0±1·01	27·0±1·75	28·8±1·15
2	26·6±0·45	28·5±0·88	27·8±1·23	24·8±0·85	25·7±1·51	27·9±0·75
3	23·2±0·59	26·0±1·03	25·9±1·00	22·9±0·90	26·3±1·18	28·3±0·70
4	21·9±0·40	24·2±0·87	25·4±0·94	24·7±0·80	27·4±1·15	29·9±0·94

The results obtained were much as could have been expected, the figures for the groups on the lower diets falling steadily as the weeks passed. By the end of the fourth week it is clear that the relationship between body weight and diet is a direct one which can be expressed by a straight line graph. This is particularly clear in the first experiment, where the starting weights of the various groups were almost equal.

Changes in liver glycogen concentration.

Since it is evident that mitotic activity is closely associated with carbohydrate concentration, the carbohydrate reserves were estimated at the close of the experiments in terms of the liver glycogen content and the blood sugar concentration. The average liver glycogen contents of the various groups of mice are shown in Table III.

TABLE III.—*The Average Liver Glycogen Concentrations (in mg. Glucose per 100 g. Fresh Weight) in Groups each of 5 Adult Male Mice Fed for 4 weeks on Restricted Diets of Rat Cake.*

Diets expressed as percentages of normal. (%)	1st experiment.	2nd experiment.
55		769·3±17·9
60	786·4±31·4	
65		846·1±28·0
70	968·5±54·7	
75		909·2±12·1
80	1011·4±50·5	
85		1013·0±17·6
90	1138·8±33·2	
95		1116·6±22·0
100	1228·5±14·8	
105		1190·9± 7·9

From these figures it is evident that the normal liver glycogen concentration in these mice was about 1200 mg. per 100 g. fresh weight. As in the case of the body weights, the reductions obtained with the restricted diets were evidently

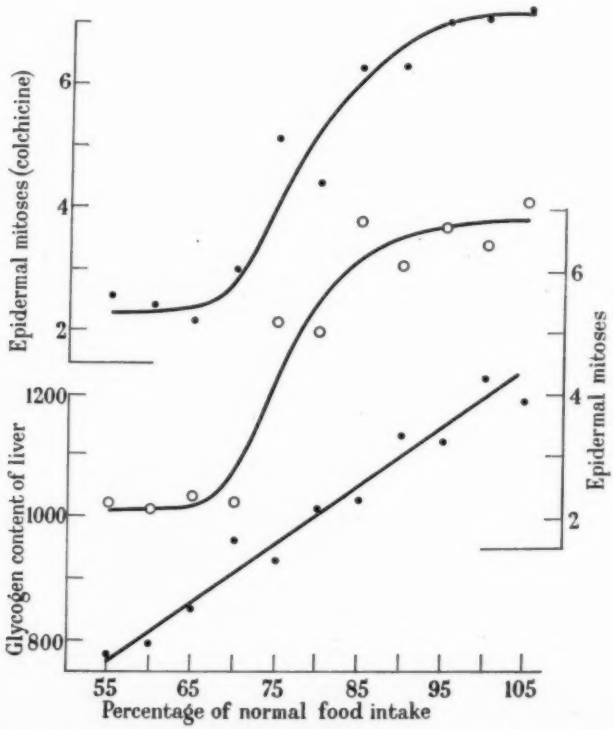

FIG. 1.—Graphs illustrating the effects of restricted diets on liver glycogen concentration and epidermal mitotic activity.

in direct proportion to the degrees of restriction, the relationship being capable of expression by means of a straight line graph (Fig. 1).

Changes in the blood-sugar level.

Evidence of reduced carbohydrate reserves following restricted diets is not so obvious from the figures for the blood-sugar concentrations recorded in Table IV.

TABLE IV.—*The Average Blood-sugar Levels (in mg. Glucose per 100 ml. Blood) in Groups each of 5 Adult Male Mice Fed for 4 weeks on Restricted Diets of Rat Cake.*

Diets expressed as percentages of normal. (%.)	1st experiment.	2nd experiment.
55		240·6±1·9
60	233·1±2·1	
65		218·1±3·3
70	214·4±4·8	
75		199·4±2·2
80	200·0±4·5	
85		187·4±1·3
90	183·4±5·9	
95		180·0±1·5
100	172·3±2·3	
105		175·1±0·8

Superficial examination of Table IV might suggest that the poorer the diet the higher the blood-sugar level. However, the higher figures recorded in the lower fed groups are doubtless due to the meal which these half-starved animals had eaten some 5 hours earlier. At that time the blood-sugar level must have been low (Bullough, 1949*b*), and with a coincidentally low secretion rate of insulin the effect of the meal must have been to raise the blood-sugar level to abnormal heights. It is thus logical that the poorer the diet the higher the blood-sugar level should be, and once again the relation is a direct one expressible as a straight line graph.

The changes in the mitoses rate.

These were worked out on the epidermis by means of the weekly removal of pieces of ear, the first pieces being taken on the day the experiment started. The results for the first experiment are shown in Table V, and those for the second in Table VI.

TABLE V.—*The Average Numbers of Mitoses Present per Unit Length (1 cm.) of Ear Epidermis (cut 7μ thick) in Groups each of 5 Adult Male Mice Fed on Restricted Diets. The figures for weeks 0 to 3 were obtained without colchicine, while that for week 4 was obtained with colchicine.*

Weeks.	Diets expressed as percentages of normal food intake.				
	60%.	70%.	80%.	90%.	100%.
0	7·6±0·30	7·0±0·19	7·2±0·20	7·0±0·25	7·3±0·30
1	2·9±0·22	2·6±0·39	5·0±0·26	6·5±0·41	7·2±0·35
2	2·2±0·21	2·4±0·23	5·0±0·51	6·7±0·28	6·6±0·17
3	2·1±0·18	2·2±0·36	5·0±0·50	6·0±0·36	6·4±0·30
4	2·4±0·19	3·0±0·24	4·3±0·29	6·2±0·10	7·1±0·35

TABLE VI.—*The Average Numbers of Mitoses Present per Unit Length* (1 *cm.*) *of Ear Epidermis* (*cut* 7μ *thick*) *in Groups each of* 5 *Adult Male Mice Fed on Restricted Diets. The figures for weeks* 0 *to* 3 *were obtained without colchicine, while that for week* 4 *was obtained with colchicine.*

Weeks.	Diets expressed as percentages of normal food intake.					
	55%.	65%.	75%.	85%.	95%.	105%.
0	7·4±0·20	7·5±0·29	7·1±0·18	7·3±0·18	7·0±0·10	7·1±0·35
1	3·6±0·36	3·6±0·42	6·0±0·39	7·4±0·36	7·6±0·39	7·3±0·44
2	2·0±0·09	2·5±0·17	5·5±0·30	6·8±0·12	7·5±0·28	7·0±0·32
3	2·2±0·18	2·3±0·18	5·2±0·32	6·9±0·22	6·7±0·34	7·1±0·41
4	2·5±0·18	2·2±0·24	5·1±0·26	6·2±0·42	7·0±0·32	7·3±0·56

One surprising conclusion that can be drawn from these figures is that the imposition of a restricted diet results in an extremely rapid fall in the mitosis rate. In most groups there was a steady reduction throughout the whole experiment, but the greatest fall occurred during the first seven days. It appears curious that the reserves of a well-fed mouse should be insufficient to prevent this.

In Fig. 1 these results are expressed graphically, the figures chosen for comparison being those of the numbers of mitoses observed after 3 weeks (without colchicine) and after 4 weeks (with colchicine). It appears that in general the mitosis rates of the lighter mice of the first experiment were lower than those of the second experiment, but the differences are remarkably slight and are of doubtful significance.

The most significant point emerging from these graphs is the indication given both with and without colchicine that the relationship between restricted diet and mitotic activity is not a direct one. The graphs are clearly sigmoid in form, the greatest drop in mitotic activity accompanying the reduction from 80 per cent to 70 per cent of the optimum diet.

DISCUSSION.

The present results confirm earlier observations that restricted diets cause a pronounced fall in mitotic activity, and indicate that when less than 70 per cent of the normal food intake is given the epidermal mitosis rate is reduced to about 35 per cent of the normal. On the basis of earlier work it can be safely suggested that this reduction is caused by a shortage of carbohydrate, in the form of glycogen or glucose, in the epidermal cells themselves (Bullough, 1949*a*, 1949*b*, 1949*c*, 1950*a*, 1950*b*; Bullough and Eisa, 1950). Evidently in conditions of semi-starvation this shortage develops very rapidly, so that the mitosis rate is almost fully depressed after only 7 days. At this time the fall in the body weight is only just beginning, and it is therefore evident that a mouse is unable to maintain a high rate of cell division by means of the utilization of fat deposits.

In relation to this point it is important to notice that in fully-fed animals the mitosis rate does not appear to vary with the body weight. In a fully-fed four-month-old mouse of about 35 g., as in a fully-fed three-month-old mouse of about 25 g., the epidermal mitosis rate reaches a sleep maximum of about 7·5 mitoses per cm. Similarly in either case the food intake remains steady at about 3·6 g. per day. When, with a lowered food intake, the body weight falls from

35 g. to 25 g. the mitosis rate is severely depressed. Clearly therefore mitotic activity is related to food intake rather than to body weight as such.

When fed on restricted diets the mice lost weight in a regular manner week by week, the speed of loss being in direct proportion to the degree of underfeeding. In a similar manner the carbohydrate reserves, as indicated by the liver glycogen content, fell after 4 weeks to a level which was directly proportional to the amount of food given.

In contrast the degree of mitotic activity seen after 3 and 4 weeks was not directly proportional to the food intake. With a reduction to 85 per cent of the

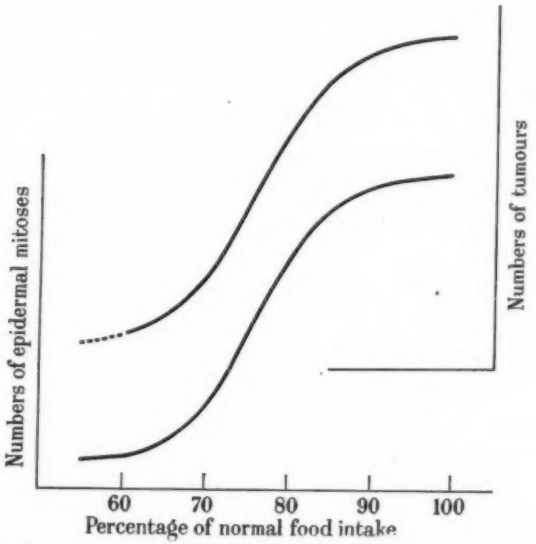

FIG. 2.—A comparison of the effects of restricted diets on epidermal mitotic activity and on carcinogenesis (Tannenbaum, 1947).

normal food intake the mitosis rate was only slightly depressed, but with a reduction from 80 per cent to 70 per cent the mitosis depression was severe. Below 70 per cent the further fall in mitotic activity was again slight. This is almost exactly similar to the result obtained by Tannenbaum (1947) when studying the effects of restricted diets on tumour genesis. His conclusions are summarized as a graph in Fig. 2, in which for comparison a graph of the present results is included. In both cases the form of the graph is sigmoid, and in both cases the maximum depression accompanies the reduction from 80 per cent to 70 per cent of the normal food intake. This adds further support to the thesis put forward by Bullough (1949*b*), and developed in the following review (Bullough, 1950*c*), that the rate of tumour genesis in a particular tissue or in the body as a whole may be directly related to the mitosis rate. The mitosis rate in turn is related to a variety of factors, of which one of the most important is the carbohydrate, or calorie, content of the food.

SUMMARY.

Groups of adult male mice were maintained for 4 weeks on diets varying from 55 per cent to 105 per cent of what they would eat if fed *ad libitum*.

Weekly records were made of the body weights and epidermal mitosis rates, and at the end of the experiments the carbohydrate reserves were estimated in terms of liver glycogen content and blood-sugar concentration.

It was found that body weight and carbohydrate reserve varied in direct proportion to the amount of food given. However, the mitosis rate did not vary directly in this way, and its relation to food intake can be expressed by means of a sigmoid curve. The greatest mitosis depression accompanied a reduction from 80 per cent to 70 per cent of the normal diet, a result which compares closely with that of Tannenbaum (1947) regarding the effects of restricted diets on carcinogenesis.

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MITOTIC ACTIVITY AND CARCINOGENESIS.

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Received for publication August 12, 1950.

IT is perhaps unnecessary to emphasize the importance of the study of cell division by mitosis. This process is one of the most fundamental of cell mechanisms, so fundamental indeed that, except in minor details, it has never been influenced by evolutionary change. Obviously the physiology of such a process is particularly fascinating, and in addition it is becoming clear that its study may shed important light on a variety of practical problems.

An intensive study of cell division has continued for a long time, having started during the last century with descriptions of the morphology of the process. However, the greater part of this work has been from the viewpoint of pathology, and has resulted on the one hand in the mass of observations on the effects of carcinogenic substances, and on the other with the discovery of the so-called mitotic poisons (Loveless and Revell, 1949). By comparison, the direct study of the physiology of normal mitosis has been surprisingly neglected, presumably because of the difficulty of the techniques involved, and because the practical value of such knowledge has not been fully appreciated.

The present discussion starts with a consideration of the physiology of normal mitosis in mammalian tissues, and continues with an examination of its relation to modern work on carcinogenesis.

GLYCOGEN AND MITOSIS.

In recent years a considerable amount of attention has been paid to the problem of mitotic activity in the epidermis of the mouse (Bullough, 1948*a*, 1948*b*, 1949*a*, 1949*b*, 1949*c*, 1949*d*, 1950*a*, 1950*b*, 1950*c*) and of the general biology of the skin (Medawar, 1947, 1948, 1949). The work on the mouse epidermis began with an analysis of the diurnal cycle of mitotic activity, which was found to be a simple rhythm directly determined by the waking and sleeping habits of the animals. During the hours of activity the mitosis rate is severely depressed, while during the hours of rest and sleep it is high. Factors determining the form of the diurnal cycle are the accustomed times of feeding, the quantity and quality of the food given, and the age and sex of the animals.

Following the discovery that muscular exercise and severe cold both depress mitotic activity, studies were made of the effect of the carbohydrate supply within the body. It was found that by means of subcutaneous injections of glucose or starch the mitosis rate can be raised in both active and sleeping mice to a level considerably higher than normal. Conversely, the depression of the

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blood-sugar level by means of injections of insulin results in a sharp reduction in the mitosis rate.

Further studies have indicated that the epidermal mitosis rate is related, not to the blood-sugar concentration, which is high during hours of activity and low during hours of sleep, but to the concentration of intracellular glycogen. Bullough and Eisa (1950) have shown that the diurnal cycle of glycogen concentration in the skin is exactly similar to the diurnal cycle of epidermal mitotic activity. It is well known that glucose is deposited from the blood during sleep, and, while most of it is stored as glycogen in the liver, it is now evident that significant quantities are also deposited elsewhere.

The next question to be considered was that of the part played by glycogen or glucose in the process of cell division. The most obvious alternatives were either that carbohydrate is incorporated, for instance as ribose, in the new nucleoplasm or cytoplasm as it is formed, or that it is destroyed to provide energy. The first alternative was rendered improbable when it was found that ribose itself has no obvious effect on epidermal mitotic activity. The second was strengthened when it was found that the stimulus obtained from extra starch can be augmented by coincident injections of phosphate, and that, conversely, mitosis can be almost eliminated by injections of phloridzin, a substance known to inhibit phosphorylation (Bullough, 1949*b*). The conclusion that mitotic activity may involve the expenditure of a significant amount of energy also receives support from the results of experiments made with dividing eggs by such men as Brachet (1932), Runnström (1933), and Zeuthen (1946, 1947, 1948). Their work suggests that the respiration rate, as measured either by oxygen intake or carbon dioxide output, rises significantly during the divisions of echinoderm and amphibian eggs.

In mammalian epidermis the importance of oxygen has been stressed by Medawar (1947) in the rabbit, and by Bullough and Johnson (Bullough, 1950*c*) in the mouse. When this tissue is kept *in vitro* anaerobic conditions inhibit cell division, and it is now clear that glycogen, phosphate and oxygen are all involved at the onset of an epidermal mitosis. However, since in a normal body it seems unlikely that either phosphate or oxygen are ever in such short supply as to become limiting factors in cell division, further elaboration of this point is not necessary here.

Diet, Mitosis and Carcinogenesis.

Summarizing what has been said above it is evident that, in a normal mouse, carbohydrate in the form of glycogen or glucose is a most important substance determining mitotic activity, and that its apparent function is to supply the energy requirements of cell division. As one outcome of these conclusions it was to be expected that diet would be found to have an important effect on the mitosis rate, and this has now been confirmed (Bullough, 1949*c*). After 12 hours' starvation the epidermal mitosis rate of a male mouse was cut by 50 per cent. after 24 hours by 75 per cent, and after 36 hours hardly any mitoses remained. In experiments involving restricted diets similar results were obtained. A normal male mouse in the presence of plenty was found to eat daily some 3·6 g. of a commercial rat cake. If kept on a 66 per cent diet of 2·4 g., which is sufficient to maintain good health, the epidermal mitosis rate fell by about 60

per cent, while on a 50 per cent diet of 1·8 g., which is insufficient, it fell by 85 per cent.

It was later confirmed that a restricted diet results in a lowered body weight, a reduced glycogen reserve, and hence a reduced mitosis rate (Bullough and Eisa, 1950). However, while the body weights and carbohydrate reserves were found to vary in direct proportion to the degree of underfeeding, the rate of epidermal mitosis varied in a complex manner expressible in terms of a sigmoid graph. It must be added that while the results primarily concerned the epidermis, other observations showed that similar conditions develop in other tissues, and it can be safely concluded that the reduction of mitotic activity by diet is a general effect visible throughout the body.

The striking thing about these observations is that they parallel so closely the results of recent work by Tannenbaum (1940*a*, 1940*b*, 1942*a*, 1942*b*, 1944*a*, 1944*b*, 1945, 1947) and Tannenbaum and Silverstone (1949*a*, 1949*b*), who have studied the effects of restricted diets on carcinogenesis. In introducing this subject it is vitally important to emphasize that it is indeed the genesis of tumours that is being considered, and not the growth of tumours once they have been formed. The growth of a visible tumour is only influenced in slight degree by variations in diet, and it is only in the process of tumour genesis that diet has any pronounced effect.

It has, in fact, been known for some time that a direct relation exists between diet, body weight, and cancer incidence. The greater part of the earlier evidence was reviewed by Hoffman (1937), who came to the conclusion that "overnutrition is common in the case of cancer patients to a remarkable and exceptional degree." This statement can be justified by figures such as those of Dublin (1929), who showed statistically that persons who are overweight during middle age have a higher expectation of death from cancer than those who remain underweight.

Now Tannenbaum, using mice, has confirmed these observations experimentally. His first experiments involved a simple restriction of diet, and led to the surprising discovery that animals maintained on a 66 per cent diet are more active, develop fewer tumours and fewer diseases, and so live longer on the average than do the fully fed controls. In later experiments all groups were provided with a basic diet of protein, fat, vitamins, and minerals, and the only differences lay in the amount of carbohydrate which they received. The same dramatic result was obtained, the animals with a restricted carbohydrate intake developing fewer tumours than those which ate their fill.

Two examples of this effect may be mentioned. Of 50 females fed *ad libitum* 29 developed spontaneous mammary tumours, while in 50 similar females fed on a 60 per cent diet no tumours appeared. Of male dba mice painted 19 times with 3:4–benzpyrene, one group of 50 fed *ad libitum* developed 32 tumours, while another group of 50 fed on a carbohydrate restricted diet developed only 11.

Similar results have been obtained with induced sarcomas, with spontaneous cancer of the lung, and with spontaneous and induced leukaemias. Indeed some ten different types of tumours have now been shown to react in this way. As a general principle it can be said that the most striking results are obtained with spontaneous tumours, which are often prevented altogether, and it is evident that with induced tumours the modifying effect of the diet can be at least partly masked if sufficiently heavy doses of carcinogens are given.

It is interesting to add that, like the mitosis rate, the tumour yield does not

bear a simple relation to the degree of underfeeding. In both cases this relation is expressible by means of a sigmoid curve, and the greatest fall in both the mitosis rate and the tumour yield accompanies a reduction of from 80 per cent to 70 per cent of the full diet.

One further important point arising from Tannenbaum's experiments is that the few tumours which do develop in the carbohydrate restricted groups do so only after unusually long intervals. Thus with an average daily intake of about 14 calories the latent period in one experiment had an average length of about 18 weeks, while with an intake of 8 calories the latent period was 39 weeks.

The general conclusions emerging from all this work on restricted diets are that carbohydrate, or calorie, shortage causes reduced body weight, a strongly depressed mitosis rate, the restriction or prevention of a wide variety of spontaneous and induced tumours, a considerable delay in the time of appearance of those few tumours which do develop, fewer diseases of all kinds, and consequently a healthier and a longer life. Clearly it is important to try to gain some insight into the mechanism whereby these effects are brought about. Tannenbaum (1947) himself has no explanation to offer, but he has rightly emphasized one crucial point that, like the depression of mitotic activity, the prevention of tumour genesis by calorie restriction is the result of a general effect operating throughout the whole body. This effect is " present in all the tissues of the body, and effective at all sites investigated."

MITOTIC ACTIVITY AND CARCINOGENESIS.

At this point it is evident that a *prima facie* case can be built up to indicate a connexion between the three factors carbohydrate lack, mitosis depression, and reduced tumour incidence. Further, it appears possible that these factors may be related to each other in this sequence, carbohydrate lack limiting mitotic activity, and low mitotic activity limiting carcinogenesis. The relation between the first and second and the first and third of these factors has been demonstrated. The evidence for the relation between the second and third, the dependence of cancer incidence on the mitosis rate, is examined below. However, before attempting this analysis it is important to consider at what possible point in the sequence of events leading to the formation of a tumour the mitosis rate may be able to exert an effect.

Theoretically following the results of Kline and Rusch (1944), Mottram (1944*a*, 1944*b*), Berenblum and Shubik (1947), and others, the formation of a visible tumour is accomplished in two main steps. In the first, whether through the action of a carcinogen, a virus, or some unknown factor, a normal cell is transformed into a cancerous cell, which then lies dormant. In the second, after a period of dormancy which may last for days, months, or years, this latent tumour cell begins to divide actively.

Of these two steps, the first need not be considered further because, although it was once suggested that the mitosis rate at the time of application of a carcinogen has an effect on the number of resulting tumours (Mottram, 1945), this theory has now been abandoned (Bilsechowsky and Bullough, 1949).

Thus it only remains to consider the process whereby the latent tumour cells are caused to emerge from their state of dormancy. It is becoming evident that this period of dormancy is a critical time in the course of events leading to the appearance of a tumour. It has been remarked by Rusch and Kline (1946) that

"during this phase there are so few neoplastic cells present that they are more or less lost among the normal cells, . . . and they must compete with the healthy cells for the nutrients in the fluids of the tissue spaces." Some of them may even succumb, and it seems highly probable that those which do survive do not begin to multiply actively until they are stimulated to do so. The theory developed below is that the stimulus to multiplication may be any one of the several factors that are known to promote cell division in normal cells, and therefore that a close and direct connexion may well exist between normal mitotic activity and carcinogenesis.

This suggestion of a connexion between the mitosis rate and the probability of tumour formation is not new. It is well known, for instance, that carcinomas, derived from mitotically active epithelia, are of much commoner occurrence than sarcomas, derived from mitotically inert connective tissues. However, it is unfortunately not yet possible to make a detailed comparison between normal mitotic activity and tumour incidence. On the one hand, the only extensive study of normal mitosis rates in a wide variety of tissues concerns the female mouse (Bullough, 1946), while on the other the only extensive analysis of the natural tumour yield tissue by tissue concerns man (Annual Statistical Reviews of the Registrar-General). However, from these two sources it is already possible to draw two broad conclusions. First, it is evident that epithelia such as those of the vagina, uterus, and rectum, which are naturally most highly active mitotically, are also most liable to develop tumours, while such mitotically inert tissues as striped muscle and brain do so rarely if at all. Second, it is obvious that while a general relation between normal mitotic activity and tumour genesis may exist, certain exceptions to the rule occur. One such exception is the duodenal mucosa, which, though it has a normal mitosis rate as high or higher than that of the rectal mucosa, shows a relatively low tumour yield. Another is the mammary gland, which, though it shows far less mitotic activity than the lining epithelium of the vagina, has a tumour yield which is relatively extremely high.

In considering these exceptions to the rule, attention must be directed to the fact that regions of pathological hyperplasia are among the commonest sites of tumour formation. Willis (1948) emphasized this strongly when he noted that "In the breast, fibro-adenomas are almost always situated in a bed of hyperplastic tissue, and persistent cystic hyperplasia is an important pre-cancerous state. In the uterus various kinds of abnormal endometrial hyperplasia are frequent, and some of these pass insensibly into carcinoma. Carcinoma of the prostate frequently arises in an organ already the seat of benign enlargement. . . . The close relationship of hepatic adenomas and carcinomas to regenerative hyperplasia is well known. In the skin, epidermal hyperplasias evoked by various irritative and inflammatory lesions sometimes become cancerous. . . . In all these cases it appears clear that the abnormal stimuli, regenerative or hormonal, which call forth hyperplastic proliferation in the tissues . . . may, should they persist, eventually evoke progressive neoplasia as well."

Incidentally this seems to be the explanation of the action of croton oil, which, after the application of a carcinogen, greatly increases the tumour yield in mice. It is a substance which has been shown to cause a local increase of as much as six-fold in the epidermal mitosis rate (Bullough, unpublished).

Here then is a possible explanation of the apparent exceptions mentioned above. Evidently, when attempting to outline a relationship between the

mitosis rate and carcinogenesis, it is necessary to bear in mind not only the normal conditions within a tissue, but also any special liability towards abnormal hyperplasia which that tissue may possess.

If now it is admitted that some connexion may be traceable between local mitotic activity and local tumour genesis, it is reasonable to consider that some connexion may also be found between the general mitosis rate of the body as a whole, and the chance that somewhere within that body a tumour may develop. It has been stressed that one factor which certainly stimulates both the general mitosis rate and the general likelihood of tumour development is an abundant supply of carbohydrate. A general rise in mitotic activity has also been described in middle-aged mice (Bullough, 1949*d*), and it has been remarked that, if such increased mitotic activity should prove to be common in mammalian middle age, it may offer some explanation for the fact that this is characteristically the cancer age.

As for the suppression of mitosis and of carcinogenesis, it is evident that both can be achieved by means of a restricted diet, and in view of the apparent role of carbohydrate during cell division, it may be expected that anything which limits the production of energy in the tissues will have a similar effect. One such substance is phloridzin, which inhibits phosphorylation, and another is dinitrophenol, which is said to uncouple the processes of phosphorylation and oxidation (Loomis and Lipmann, 1948). As regards phloridzin, Bullough (1949*b*) has described its effect in depressing the mitosis rate, and he has also obtained results showing a lowered yield of spontaneous mammary tumours. As regards dinitrophenol, Clowes and Krahl (1936) first noticed its power to inhibit mitosis, and Tannenbaum and Silverstone (1949*b*) have recently shown that it, too, reduces the yield of spontaneous mammary tumours.

Environmental cold and muscular exercise are also known to depress mitotic activity, apparently by diverting to other uses the energy produced from carbohydrate (Bullough, 1949*a*). Tannenbaum and Silverstone (1949*b*) have now shown that cold has a similar depressing effect on carcinogenesis, but the effect of prolonged muscular exercise has apparently not yet been determined.

All this evidence supports the hypothesis that a high mitosis rate is associated with a high tumour yield, and a low mitosis rate with a low tumour yield. Clearly, however, as was pointed out above, those conditions which stimulate hyperplasia cannot of themselves result in the formation of cancerous cells. They merely provide a suitable environment for the active multiplication of any latent tumour cells which may be present. Thus the explanation of the connection between hyperplasia and cancer may rest quite simply with the fact that with a higher mitosis rate there is a correspondingly higher chance that some latent tumour cell will be stimulated to multiply, an effect which will become evident both in the earlier development of tumours, and in the appearance of many which would otherwise never have formed. Conversely, hypoplasia may be expected to reduce the chances of multiplication, and so, as Tannenbaum's (1947) results show, to delay the development of those few tumours which do form and to prevent the appearance of many which otherwise would have formed.

SUMMARY AND CONCLUSIONS.

Evidence has been reviewed to indicate the close dependence of both mitotic activity and tumour genesis on the carbohydrate, or calorie, supply. In analysing

this conclusion it has been suggested that an abundance of carbohydrate stimulates only mitotic activity, and that the observed effect on carcinogenesis is in fact due to the raised mitosis rate. It appears possible that the average length of the period of dormancy, which every newly-formed tumour cell appears to experience, is determined by the mitosis rate of the tissue in which the cell lies. The mitosis rate itself is determined by a variety of factors, local and general, normal and abnormal, of which one is the carbohydrate, or calorie, supply.

This conclusion is in agreement with a suggestion that the practical problem of cancer can be divided into two distinct parts: the formation of the latent tumour cell, and the breaking of its period of dormancy. The question posed by the first of these is still far from being answered, but the question posed by the second may be much simpler. Already it is known that the period of dormancy can be lengthened simply by means of a restricted diet, and with a more detailed understanding of those factors which control normal mitotic activity other practical methods may be devised.

These conclusions are based mainly on the study of the mouse, but there seems no reason to doubt that they will be found equally applicable to man. Already it is known that the incidence of human cancer varies in direct proportion to body weight, and therefore presumably to food intake, and it does not seem impossible that the idea of strict dieting to maintain the body weight of the middle-aged at an optimum level may some day be an accepted and normal practice.

As a postscript, attention may be drawn once more to Tannenbaum's (1947) observation that with restricted diets disease incidence as well as tumour incidence is markedly lowered. In considering this further question it seems possible that parasites penetrating into a tissue may, like the latent tumour cells, have to compete with the normal cells for the available nutrients. If these nutrients are in short supply the multiplication of the parasites may be impeded or even prevented. Already it is known that in conditions of hypoglycaemia canaries are less liable to contract malaria (Hegner, 1937) and rats are less liable to contract tuberculosis (Steinbach and Duca, 1942). It now appears that the whole subject of competition between cells, and perhaps also between tissues, is worthy of the most serious consideration for the practical results which it may yield.

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THE ABSORPTION OF URETHANE FROM MOUSE SKIN.

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Received for publication September 4, 1950.

URETHANE causes a very marked depression of mitotic activity of certain mouse, rat and rabbit cells (Dustin, 1947 ; Guyer and Claus, 1947). The present work was undertaken to determine whether any such action would affect the process of malignant transformation as brought about by painting a carcinogenic hydrocarbon on mouse skin.

MATERIAL AND METHODS.

The carcinogen used was 1:2:5:6-dibenzanthracene and the urethane was applied simultaneously. Young adult stock laboratory male mice were divided into three groups and treated with solutions as shown in Table I.

TABLE I.—*Number of Mice in Each Group and their Treatment.*

Group.	Number of mice.	
1	24	20 per cent urethane (w/v) in a saturated solution of 1:2:5:6-dibenzanthracene in acetone.
2	24	Saturated solution of 1:2:5:6-dibenzanthracene in acetone.
3	18	20 per cent urethane in acetone (w/v).

A drop of the solution was applied interscapularly on the skin of the mice, by paint-brush, once a week, for one year till the mice died or were sacrificed. In this way a local and simultaneous action of urethane and hydrocarbon on mouse skin was obtained (Group 1). Group 2 served as a control, receiving the hydrocarbon only. Group 3 was given urethane alone in order to observe any independent effects which it might cause over a long period of time.

RESULTS.

Most of the mice in Groups 1 and 2 epilated at the site of application and developed papillomas which eventually became malignant. The time taken for these processes varied greatly between individual mice of both Groups 1 and 2, but no group difference was noted. It was difficult to determine exactly when a papilloma became malignant, but once the malignant process began, the epitheliomas grew at a fairly constant rate. The best criterion of response to treatment was considered to be the time taken for an epithelioma to reach a

given size, in this case 1 cm. in diameter. This was found to be quite practical, since all the tumours were nearly circular in plan and had a fairly discrete edge. When the tumour reached this size the mouse was sacrificed and a post-mortem examination made.

In the early part of the experiment a high mortality was noted in mice of Groups 1 and 3. In most cases post-mortem changes in the mice had proceeded to an extent which made diagnosis of cause of death impossible. Occasionally ectromelia and mouse typhoid was noted and the deaths were attributed to general infections. Fig. 1 shows the progress of the experiment. The increased mortality in Groups 1 and 3 is obvious from Fig. 1. At the end of the experiment one mouse

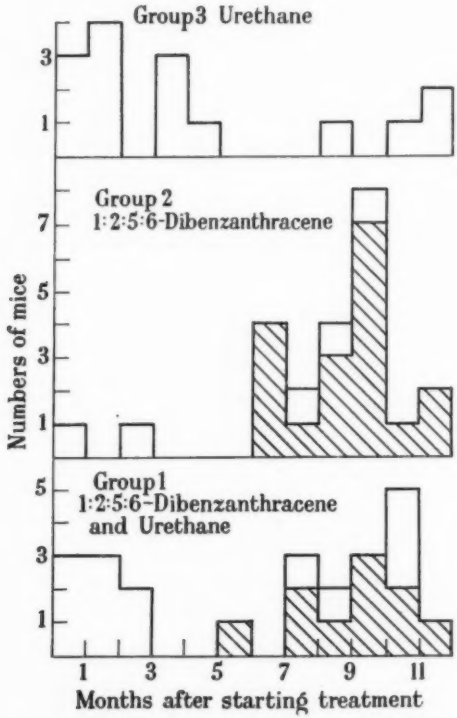

FIG. 1.—Mortality and tumour development rates of mice painted with 1:2:5:6-dibenzanthracene, urethane, and both. The plain squares represent mice which died of infection, and the shaded squares represent those mice which were killed while bearing a 1 cm. diameter epithelioma.

in each of Groups 1 and 2 was alive and bearing non-malignant papillomas, and three mice in Group 3 were alive.

As can readily be seen from Fig. 1, the incidence and time of onset of epitheliomas was the same in mice of Groups 1 and 2.

When the first mouse with epithelioma in Group 1 was sacrificed and examined it was found to contain multiple lung tumours. This mouse had been treated

for 5–6 months. Macroscopically and microscopically the lung tumours were identical in appearance with those induced by the injection of urethane (Nettleship and Henshaw, 1943). It was found that all mice in Groups 1 and 3 after six or more months' treatment, had developed multiple pulmonary adenomas which appeared to be typically urethane-induced. No mice in Group 2 showed even a single macroscopic lung tumour. Apart from skin and lung tumours, none of the mice sacrificed with epitheliomas showed any other obvious pathological condition.

DISCUSSION.

The results show that, under the experimental conditions described here, urethane causes no obvious change in the frequency or time of appearance of papillomas or epitheliomas induced by 1:2:5:6-dibenzanthracene. It is of course possible that urethane might have influenced the development of skin tumours under different conditions. Tannenbaum and Silverstone (1947) have pointed out that massive doses of carcinogens tend to mask therapy effects. Consequently a better test to detect a weak inhibiting activity of urethane might have consisted of treating mouse skin with one application of the hydrocarbon, and thereafter more frequent urethane applications.

Furthermore the information gained is applicable to the end result. It remains possible that the details of the processes were not identical. No studies were made of the histological effects of urethane on either normal skin or skin undergoing malignant transformation, since the experiments suggest that any such effects have little action on the production of cancer.

It is most interesting to note that those mice which received urethane treatment (Groups 1 and 3) had multiple lung tumours, whereas those receiving 1:2:5:6-dibenzanthracene alone (Group 2) had none. The possibility that the mice licked the urethane from each other's skin was considered to be most unlikely. When urethane was administered to mice in their drinking water in previous experiments, a reduced fluid consumption was noted for the first few days, showing their aversion to it. It seemed therefore that the urethane, after application to the skin, was absorbed by the mice in sufficient quantities to induce lung adenomas. An estimate was made of amount of urethane in a paint-brushfull of the solution (as painted on the mice) by applying it to a cover slip and weighing after the acetone had evaporated. Approximately 2 mg. was found to be thus deposited. Assuming all the urethane to be absorbed by the mouse, this treatment is equivalent to the injection of 0·2 c.c. of a 1 per cent urethane solution into the mice each week. For previous studies (Cowen, 1947), lung tumours in comparable numbers to those obtained here were induced in various inbred lines of mice by injecting 0·25 c.c. of a 10 per cent solution weekly for $3\frac{1}{2}$ months, and then killing the mice after a further 4 months. Since total dosage was not the same, and since there are marked strain differences with respect to lung tumour response by different lines of mice, no accurate comparison can be made. It would be safe to say, however, that a large part of the urethane applied to the skin was absorbed by the mice in this experiment, judging by their tumour response.

A further indication that the urethane was absorbed is given by the mortality of the mice in Groups 1 and 3, but not in Group 2. It has previously been shown that mice suffer an increased mortality when given urethane either by injection

(Cowen, 1947) or in the drinking water (Cowen, 1950). The high death rate noted here is considered to be related to the treatment with urethane.

It is not unreasonable to suppose that urethane is absorbed at least as readily from human as from mouse skin. Since urethane causes leucopenia in man (Moeschlin and Meili, 1947) and lung cancer in mice (Nettleship and Henshaw, 1943) and rats (Jaffé, 1947), it would be well for those who handle this substance frequently in the laboratory or commercially to consider it a possible risk to health and consequently take effective precautions.

SUMMARY.

Three groups of mice were painted interscapularly with an acetone solution of 1:2:5:6-dibenzanthracene, urethane and a mixture of both each week.

The groups receiving the hydrocarbon or the mixture developed the same incidence of epitheliomas and at the same rate.

The groups receiving urethane or the mixture developed multiple pulmonary adenomas and showed a high mortality rate.

This points out that most of the urethane applied to the skin was absorbed by it.

All expenses in connection with this work were borne by the British Empire Cancer Campaign.

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THE EFFECT OF URETHANES AND DI(2-CHLOROETHYL) METHYLAMINE HYDROCHLORIDE ON THE AUTOXIDATION OF DOPA.

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Received for publication June 10, 1950.

URETHANE (ethylcarbamate) has recently received much attention because of its use in the treatment of chronic myeloid leukaemia and other members of the group of diseases of doubtful nature at present called "reticuloses," and also because of its action as a carcinogen. The inhibitory effect of urethane on dehydrogenases has long been known, but apparently its action on other types of enzymes has not been investigated in detail. It was therefore thought to be of interest to study the action of urethane on the oxidation of tyrosine, by tyrosinase, to melanin.

Tyrosine solution of concentration 0·02 per cent in buffer pH 7·4 was prepared, and ethyl carbamate solution of 10 per cent concentration in the same buffer. The enzyme used was crude tyrosinase from potatoes, freshly prepared for each experiment. A typical experimental lay-out is shown in Table I.

TABLE I.—*Typical Experiments.*

Flask No.:—	1.	2.	3.	4.	5.	6.
	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
Tyrosine solution	10	10	10	10	10	10
Urethane solution	10	8	6	4	2	0
Buffer solution	0	2	4	6	8	10
Tyrosinase solution	1	1	1	1	1	1
Urethane per cent	5	4	3	2	1	0
Tyrosine per cent	0·01	0·01	0·01	0·01	0·01	0·01

By this method all flasks contained the same volume of solution, Flask 6 acting as control. The flasks used were all of the same shape and size. The flasks were placed in a Warburg bath at a temperature of 37·5° C. and shaken with a stroke of 6 cm. at a rate of 90 per minute. Melanin formation was estimated by observing the extinction coefficients of the solutions at appropriate intervals, using an E.E.L. electro-colorimeter with tricolour green filter.

A marked inhibition of melanin formation was observed in the solutions containing urethane, the degree depending on the concentration of this latter substance. The upper limit of urethane concentration investigated was 5 per cent; the lower limit at which inhibition could be detected by the method employed was 0·2 per cent.

From what is known of the chain of events in the oxidation of tyrosine to melanin (Raper, 1926), it will be evident that this finding does not provide specific evidence for an action of the urethane on the enzyme. The first step in this oxidation is the conversion of tyrosine into 3, 4-dihydroxyphenylalanine (dopa),

TABLE II.—*The Oxidation of Tyrosine to Melanin in the Presence of* 5, 4, 3, 2, 1 *and* 0 *per cent Ethyl Carbamate* (17.vi.48).

Values of "E."

Urethane per cent:— Time.	5.	4.	3.	2.	1.	0.
22.00	·0269	·0269	·0292	·0246	·0315	·0292
22.30	·0292	·0292	·0315	·0269	·0339	·0386
23.00	·0362	·0362	·0410	·0386	·0458	·0531
23.30	·0386	·0386	·0434	·0410	·0506	·0630
00.00	·0410	·0410	·0458	·0434	·0531	·0655
01.00	·0410	·0434	·0482	·0458	·0555	·0655
02.00	·0434	·0458	·0506	·0482	·0580	·0706

Increments of "E."

30	·0023	·0023	·0023	·0023	·0024	·0094
60	·0093	·0093	·0118	·0140	·0143	·0239
90	·0117	·0117	·0142	·0164	·0191	·0338
120	·0141	·0141	·0166	·0188	·0216	·0363
180	·0141	·0165	·0190	·0212	·0240	·0363
240	·0165	·0189	·0214	·0236	·0265	·0414

and it is for this step only that the enzyme is essential. The further oxidation of dopa to melanin can occur without the aid of the enzyme, though the enzyme produces marked acceleration of the process, and the evidence so far given throws no light on which step in the oxidation is inhibited. The effect of urethane on the autoxidation of dopa was therefore investigated.

The method used was identical with that for tyrosine, with the exceptions that the final concentration of dopa was 0·005 per cent, and the absence of the enzyme. Particular care was taken to check the pH of the mixed solutions both before and after the oxidation. No evidence of any shift in pH could be detected. A marked acceleration in the production of melanin was found in the solutions containing urethane, the degree increasing with the concentration of this latter substance.

TABLE III.—*The Autoxidation of Dopa in the Presence of* 5, 4, 3, 2, 1 *and* 0 *per cent. of Ethyl Carbamate.*

The mean values for three experiments on 29.vi.48, 1.vii.48 and 25.vii.48.

Increments of "E."

Urethane per cent:— Time.	5.	4.	3.	2.	1.	0.
60	·1120	·0952	·0901	·0893	·0860	·0809
120	·3692	·3298	·3138	·3012	·2866	·2686
180	·6105	·5510	·5152	·4873	·4639	·4257
240	·8377	·7652	·7133	·6591	·6282	·5810

From this it appears probable that urethane affects the tyrosine-tyrosinase reaction in two ways: it inhibits the enzymatic conversion of tyrosine to dopa, and it accelerates the conversion of dopa to melanin. It seemed that further information might be obtained by investigating the effect of urethane on the enzymatic oxidation of dopa. The method used was again as for the non-enzymatic oxidation of dopa, with the addition of 1 c.c. of crude tyrosinase to each flask.

The results obtained showed a small degree of inhibition in the solutions containing urethane during the first 1 to 2 hours, and a small degree of acceleration during the final 2 hours. This was at first attributed to experimental errors, but the experiment was made 8 times, each time with the same result. On the assumption that the errors inevitably present are to be expected to operate equally in either direction, the probability of obtaining 8 results the same is only 1 in 128. It therefore appears that these results are to be regarded as representing the true state of affairs.

TABLE IV.—*The Enzymatic Oxidation of Dopa in the Presence of* 0, 1, 2, 3 *and* 4 *per cent of Ethyl Carbamate* (2.x.48).

	Increments of "E."				
Urethane per cent :— Time.	0.	1.	2.	3.	4.
60	·1496	·1427	·1367	·1405	·1427
120	·2735	·2757	·2716	·2818	·2798
180	·3714	·3768	·3768	·3957	·3925
240	·4663	·4750	·4815	·4995	·5017

The inhibition of tyrosinase by urethane is, in view of the previously known action of this substance, not surprising. Presumably the enzyme combines with the urethane. The acceleration of the autoxidation of dopa by urethane was unexpected, and at this stage no explanation of this can be offered. The autoxidative processes do not seem to have received the same attention as have the enzymatic ones, and the author has been unable to find any references in the literature to substances affecting their speeds, other than those producing marked changes in pH. Particular attention has been directed to this point, and no change in pH could be detected (phenyl red indicator, compared in electrocolorimeter). But a most interesting point arises from the enzymatic oxidation of dopa in the presence of urethane.

It is well known that tyrosinase accelerates the oxidation of dopa, i.e. the enzymatic oxidation of dopa proceeds very much faster than does the autoxidation. Yet at the commencement of the reaction there was inhibition in the solution containing urethane. If the urethane combines with the enzyme, and the urethane and enzyme are present in such concentrations that they neutralize each other, there should be no difference between the reaction speeds of this solution and the control. If there is excess of urethane, then this excess should operate to accelerate the reaction. If there is excess of enzyme, then again the excess should operate to accelerate the reaction. But in fact the contrary was observed, and the position is further complicated by the acceleration of the urethane-containing solution in the later stages of the reaction. The only suggestion which at present appears to cover these observations is that urethane has a greater attraction for the enzyme than has dopa, and that one or more of the first reaction products of their interaction acts as an inhibitor of the autoxidation of dopa. Later, when the enzyme has disposed of the urethane, and the intermediate metabolites have undergone further change to substances which presumably do not affect the progress of the oxidation of dopa, dopa is acted on by the enzyme, hence the acceleration of the reaction in its terminal stages.

It must be emphasized that this explanation is purely hypothetical; no direct evidence for the combination of tyrosinase with urethane, or of the presence of reaction products, either intermediate or final (other than melanin), has been obtained. Nevertheless it does appear than in the enzymatic oxidation urethane plays the part of an antoxidant.

At this stage the possibility of using these results to give an explanation of the action of urethane in leukaemia was considered. It is not suggested that the oxidation of tyrosine to melanin has any place in either the normal or abnormal metabolism of leucocytes, though there is the interesting fact that these cells do contain a polyphenolase capable of oxidizing dopa to melanin. But it did seem possible that other similar oxidations might be involved. Haddow and Sexton (1946) state that only ethyl carbamate, out of various similar substances tested, has any appreciable effect in leukaemia. Methyl, propyl and butyl carbamates were prepared, the choice being limited to these as they are freely water-soluble, and their actions could be investigated by the method used for ethyl carbamate, and compared directly with that substance.

Solutions of the four carbamates were prepared in equimolecular proportions, the experimental procedure being as before except that the concentration of dopa was 0·001. When this work was first started concentrations of 0·02 per cent of dopa and tyrosine were used. It has been found advantageous to reduce these until the present level of 0·001 per cent has been reached, as these lower concentrations prolong the period during which melanin formation can be estimated by the colorimeter. All the solutions inhibited the tyrosine-tyrosinase reaction, no significant difference being observed between the different carbamates. But when the effects on the autoxidation of dopa were investigated, it was found that ethyl carbamate produced a greater degree of acceleration than any of the others. The concentrations used were methyl carbamate 4·000 per cent, ethyl carbamate 4·747 per cent, propyl carbamate 5·494 per cent, butyl carbamate 6·240 per cent.

TABLE V.—*Autoxidation of Dopa in the Presence of Methyl, Ethyl, Propyl and Butyl Carbamates in Equimolecular Proportions* (25.v.50).

	Increments of "E."				
Carbamate :— Time.	None.	Methyl.	Ethyl.	Propyl.	Butyl.
60	·0450	·0500	·0557	·0508	·0554
120	·1242	·1332	·1475	·1357	·1435
180	·2063	·2211	·2396	·2218	·2303
240	·2855	·3078	·3316	·3056	·3195
300	·3613	·3879	·4177	·3866	·4083

It therefore appears that the difference in the action of these carbamates is not in their effect on enzymatic oxidation, but on autoxidation. The difference, however, is not great, and certainly does not seem to be of sufficient magnitude to allow of any explanation of the known action on leukaemia. It was thought possible that the effect of another water-soluble carcinogen and chemotherapeutic agent might throw further light on the problem. The effect of di(2-chloroethyl) methylamine hydrochloride on the oxidation of tyrosine was investigated. The nitrogen mustard was used in strengths up to 0·2 per cent, and at this concentration

had no effect whatever on the enzymatic oxidation of tyrosine. The effect on the autoxidation of dopa was then investigated. The lowest concentration used was 0·05 per cent, and at this strength a marked effect was produced. For approximately the first 2 hours the oxidation in the solution containing the nitrogen mustard was accelerated ; for the second 2 hours retarded. The experiment was performed 8 times, with the same result, thus establishing statistical significance. For the last of these experiments readings were taken every 15 minutes until the change from acceleration to retardation occurred. It is of particular interest to compare the differences between these two solutions, when it will be noticed that the changes are, within the limits of accuracy of the method, very regular.

TABLE VI.—*Effect of Di(2-chloroethyl) Methylamine Hydrochloride on the Autoxidation of Dopa* (13.iv.50).

Solution A, control. Solution B, plus nitrogen mustard.

	Increments of "E."		
Time.	Solution A.	Solution B.	B—A.
15	·0066	·0089	·0023
30	·0133	·0204	·0071
45	·0271	·0322	·0051
60	·0414	·0443	·0029
75	·0586	·0517	—·0069
90	·0791	·0618	—·0172
120	·1234	·0774	—·0260
180	·2174	·1019	—·1155
240	·3099	·1220	—·1879

The suggested explanation in this case is that an intermediate metabolite of the interaction between nitrogen mustard and dopa gradually accumulates until it reaches such a concentration that it inhibits the reaction, thus masking the accelerating effect of the nitrogen mustard.

It therefore appears that there is a similarity of action between di(2-chloroethyl) methylamine hydrochloride and the urethanes, and that this is in their action on autoxidation and not on enzymatic oxidation. It is obvious that it would be quite unjustifiable to attempt to explain the action of these substances in the leukaemias and other reticuloses on the grounds of their effects on melanin formation. On the other hand, evidence is produced for one action in which these substances of greatly different chemical constitution behave in a similar manner. In view of our present lack of knowledge of the reticuloses, and especially because of their almost inevitably fatal nature, it might be of considerable interest and possibly of value to investigate the effect on these diseases of other groups of substances which accelerate the progress of autoxidations. The author has been unable to obtain any information on such substances.

SUMMARY.

Ethyl carbamate inhibits the enzymatic oxidation of tyrosine, but accelerates the autoxidation of dopa. It has at first an inhibiting and later an accelerating action on the enzymatic oxidation of dopa.

Methyl, propyl and butyl carbamates have the same effects, but to a lesser degree.

The water-soluble nitrogen mustard, di(2-chloroethyl) methylamine hydrochloride does not inhibit the enzymatic oxidation of tyrosine in a concentration of 0·2 per cent. It first accelerates and then retards the autoxidation of dopa.

Suggestions for the explanation of these findings are made.

It is suggested that other substances affecting autoxidations in a similar manner might produce effects in the reticuloses.

The work has been carried out with the aid of a grant from the Government Grant Committee of the Royal Society.

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